

09/334488

PTO/SB/81A (12-08)

Approved for use through 11/30/2011. OMB 0851-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT – POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS		Patent Number		6,214,865	
		Issue Date		April 10, 2001	
		First Named Inventor		Bruce A. Littlefield	
		Title	MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND		
		Attorney Docket No.		029163.0022-US01	

I hereby revoke all previous powers of attorney given in the above-identified patent.

☐ A Power of Attorney is submitted herewith.
OR
☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

26853

OR
☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number	Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified patent to:

☒ The address associated with the above-mentioned Customer Number.
OR
☐ The address associated with Customer Number:

26853

OR

☐ Firm or Individual Name

Address

City

State

Zip

Country


Telephone

Email

I am the:

☐ Inventor, having ownership of the patent.
OR
☒ Patent owner.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on (submitted herewith)

SIGNATURE of Inventor or Patent Owner

Signature		Date	02/08/2011
Name	Nobuo Deguchi	FC:1457	De 4220/00-DA-29 2010
Title and Company	President — Eisai R&D Management Co., Ltd.		

NOTE: Signatures of all the inventors or patent owners of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ Total of 1 forms are submitted.

02/08/2011 RLOGAN 00000002 500740 09/334488

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STATEMENT UNDER 37 CFR 3.73(b)Applicant/Patent Owner: Bruce A. Littlefield, et al., / Eisai R&D Management Co., Ltd.Application No./Patent No.: 09/334,488 / 6,214,865 Filed/Issue Date: June 16, 1999 / April 10, 2001Titled: MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATIONEisai R&D Management Co., Ltd., a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;
2. ☐ an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or
3. ☐ an assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)
the patent application/patent identified above by virtue of either:

- A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

OR

- B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Bruce A. Littlefield, et al. To: Eisai Co., Ltd.
The document was recorded in the United States Patent and Trademark Office at
Reel 010118, Frame 0278, or for which a copy thereof is attached.
2. From: Eisai Co., Ltd. To: Eisai R&D Management Co., Ltd.
The document was recorded in the United States Patent and Trademark Office at
Reel 020352, Frame 0458, or for which a copy thereof is attached.
3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

- ☒ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.


Signature

Nobuo Deguchi
Printed or Typed Name

December 29, 2010
Date

President
Title

Effective on 12/08/2004. Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818). FEE TRANSMITTAL For FY 2009		Complete if Known	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27		Application Number	Patent No.: 6,214,865
		Filing Date	Issued: April 10, 2001
		First Named Inventor	Bruce A. Littlefield
		Examiner Name	N/A
		Art Unit	N/A
TOTAL AMOUNT OF PAYMENT		(\$) 1,120.00	
		Attorney Docket No.	029163.0022-US01

RECEIVED

METHOD OF PAYMENT (check all that apply)	
<input type="checkbox"/> Check <input type="checkbox"/> Credit Card <input type="checkbox"/> Money Order <input type="checkbox"/> None <input type="checkbox"/> Other (please identify): _____	
<input checked="" type="checkbox"/> Deposit Account Deposit Account Number: <u>50-0740</u> Deposit Account Name: <u>Covington & Burling</u>	
For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)	
<input checked="" type="checkbox"/> Charge fee(s) indicated below	<input type="checkbox"/> Charge fee(s) indicated below, except for the filing fee
<input checked="" type="checkbox"/> Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17	<input checked="" type="checkbox"/> Credit any overpayments

JAN 11 2011

PATENT EXTENSION
 OPLA

FEE CALCULATION							
1. BASIC FILING, SEARCH, AND EXAMINATION FEES							
Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	
Utility	330	165	540	270	220	110	
Design	220	110	100	50	140	70	
Plant	220	110	330	165	170	85	
Reissue	330	165	540	270	650	325	
Provisional	220	110	0	0	0	0	
2. EXCESS CLAIM FEES							
						Small Entity	
						Fee (\$)	Fee (\$)
Each claim over 20 (including Reissues)						52	26
Each independent claim over 3 (including Reissues)						220	110
Multiple dependent claims						390	195
Total Claims		Extra Claims	Fee (\$)	Fee Paid (\$)	Multiple Dependent Claims		
- 20 or HP		x	=		Fee (\$) Fee Paid (\$)		
HP = highest number of total claims paid for, if greater than 20.							
Indep. Claims		Extra Claims	Fee (\$)	Fee Paid (\$)			
- 3 or HP =		x	=				
HP = highest number of independent claims paid for, if greater than 3.							
3. APPLICATION SIZE FEE							
If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$270 (\$135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).							
Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)			
- 100 =	/50 =	(round up to a whole number) x	=				
4. OTHER FEE(S)							
Non-English Specification, \$130 fee (no small entity discount)							
Other (e.g., late filing surcharge): 1457 Extension of term of patent						1,120.00	

SUBMITTED BY			
Signature		Registration No. (Attorney/Agent)	36,744
Name (Print/Type)	Paul J. Berman	Telephone	(202) 662-5468
		Date	January 11, 2011

Docket No.: 029163.0022-US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Letters Patent of:
Bruce A. Littlefield, *et al.*

Patent No.: 6,214,865

Issued: April 10, 2001

For: MACROCYCLIC ANALOGS AND METHODS
OF THEIR USE AND PREPARATION

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**PATENT EXTENSION
OPLA**

TRANSMITTAL LETTER

Mail Stop: Hatch-Waxman PTE
U.S. Patent and Trademark Office
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Dear Sir:

Enclosed are the following items for filing in connection with the above-referenced issued Patent:

1. Fee Transmittal;
2. Application for Extension of Patent Term Under 35 U.S.C. § 156, together with Exhibits 1-9 (original plus two (2) copies);
3. Pursuant to M.P.E.P. § 2753, an additional two (2) copies of this Application, together with Exhibits 1-9, are included for public inspection in the Office of Patent Legal Administration, and for use by the Legal Advisor;
4. Pursuant to 37 C.F.R. § 1.740(14), one (1) copy of this Application (without exhibits) is included for fee purposes;

5. Patent — Power of Attorney or Revocation of Power of Attorney with a New Power of Attorney and Change of Correspondence Address;
6. Statement Under 37 C.F.R. 3.73(b); and
7. One stamp and return receipt postcard.

The Director is authorized to charge our Deposit Account No. 50-0740 in the amount of \$1,120.00 to cover the fee for a request for extension of patent term. The Director is also authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to prevent this application from being inadvertently abandoned to our Deposit Account No. 50-0740, under Docket No. 029163.0022-US01. A duplicate copy of this paper is attached.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned, and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 50-0740.

Dated: January 11, 2011

Respectfully submitted,

By 

Paul J. Berman

Registration No.: 36,744

Christopher N. Sipes

Registration No.: 39,837

COVINGTON & BURLING LLP

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Washington, DC 20004-2401

Telephone No.: 202.662.6000

Facsimile No.: 202.662.6291

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Letters Patent of:
Bruce A. Littlefield, *et al.*

Patent No. 6,214,865

Issued: April 10, 2001

For: MACROCYCLIC ANALOGS AND METHODS
OF THEIR USE AND PREPARATION

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Mail Stop: Hatch-Waxman PTE
U.S. Patent and Trademark Office
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

APPLICATION FOR EXTENSION OF PATENT TERM UNDER
35 U.S.C. § 156

Dear Sir:

Pursuant to 35 U.S.C. § 156 and 37 C.F.R. §§ 1.710–1.791, Eisai R&D Management Co., Ltd., (“Applicant”), herewith applies for an extension of the term of U.S. Patent No. 6,214,865 (Exhibit 1, the “’865 patent”).

The Applicant represents that its address is 6-10 Koishikawa 4-chome, Bunkyo-ku, Tokyo, Japan 112-8088, and that it is the owner and assignee of the entire interest in and to Letters Patent of the United States No. 6,214,865, granted to Bruce A. Littlefield, Monica H. Palme, Boris M. Seletsky, Murray J. Towle, Melvin J. Yu, and Wanjun Zheng on April 10, 2001, for “Macrocyclic Analogs and Methods of Their Use and Preparation,” by virtue of an assignment from Eisai Co., Ltd. This assignment was recorded in the U.S. Patent and Trademark Office (“USPTO”) on January 11, 2008, at Reel 020352, Frame 0458 and is attached hereto as Exhibit 2. Eisai Co., Ltd. had been the owner of the ’865 patent by virtue of an assignment from

Bruce A. Littlefield, Monica H. Palme, Boris M. Seletsky, Murray J. Towle, Melvin J. Yu, and Wanjun Zheng of their interests in U.S. Patent Application No. 09/334,488 (the “’488 application”), recorded on July 26, 1999, at Reel 010118, Frame 0278, and attached hereto as Exhibit 2. The ’865 patent matured from the ’488 application, filed on June 16, 1999, which claims priority under 35 U.S.C. § 119(e) to Provisional Application No. 60/089,682, filed on June 17, 1998.

The Approved Product that is relevant to this application is HALAVEN™ (eribulin mesylate) Injection, 1 mg/2 mL (0.5 mg/mL), referred to herein as “HALAVEN™” or “Approved Product.”

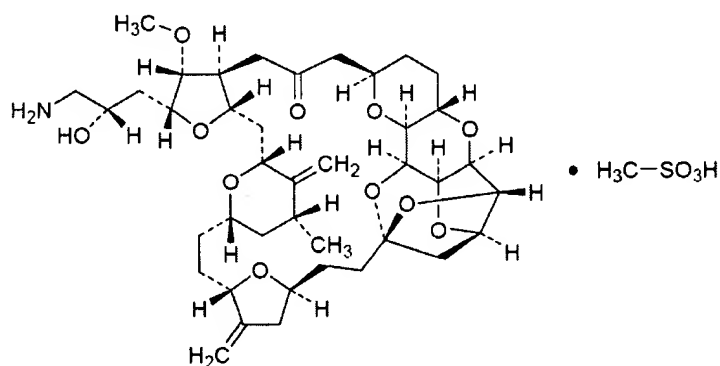
The Marketing Applicant for HALAVEN™ is Eisai Inc., located at 100 Tice Boulevard, Woodcliff Lake, NJ 07677. The Marketing Applicant is a wholly-owned subsidiary of Eisai Corporation of North America, which in turn is a wholly-owned subsidiary of Eisai Co., Ltd. Eisai R&D Management Co., Ltd., the owner of the ’865 patent, is also a wholly-owned subsidiary of Eisai Co., Ltd. Additionally, the initial IND applicant, Eisai Medical Research Inc., was a wholly-owned subsidiary of Eisai Corporation of North America, which in turn was and is a wholly-owned subsidiary of Eisai Co., Ltd. Eisai Medical Research Inc. was merged into, and sponsorship of the IND was transferred to, Eisai Inc., on October 1, 2009.

The following information is submitted by Applicant in accordance with 35 U.S.C. § 156(d) and the rules for extension of patent term issued by the USPTO at 37 C.F.R. Subpart F, §§ 1.710 to 1.791, and follows the numerical format set forth in 37 C.F.R. § 1.740.

(1) A COMPLETE IDENTIFICATION OF THE APPROVED PRODUCT AS BY APPROPRIATE CHEMICAL AND GENERIC NAME, PHYSICAL STRUCTURE OR CHARACTERISTICS:

The Approved Product is HALAVEN™, with the active ingredient eribulin mesylate at a strength of 1 mg per 2 mL (0.5 mg per 1 mL). The Food and Drug Administration (“FDA”) has approved HALAVEN™ for intravenous administration for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting. The approved labeling is attached hereto as Exhibit 3.

The chemical name of eribulin mesylate is 11,15:18,21:24,28-Trieпоxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one, 2-[(2*S*)-3-amino-2-hydroxypropyl]hexacosahydro-3-methoxy-26-methyl-20,27-bis(methylene)-, (2*R*,3*R*,3*aS*,7*R*,8*aS*,9*S*,10*aR*,11*S*,12*R*,13*aR*,13*bS*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29*aS*)-, methanesulfonate (salt), with the chemical structure:



Its molecular formula is $C_{40}H_{59}NO_{11} \cdot CH_4O_3S$, and its molecular weight is 826.0 (729.9 for free base).

HALAVEN™ is a clear, colorless, sterile solution for intravenous administration. Each vial contains 1 mg of eribulin mesylate as a 0.5 mg/mL solution in ethanol:water (5:95).

(2) A COMPLETE IDENTIFICATION OF THE FEDERAL STATUTE INCLUDING THE APPLICABLE PROVISION OF LAW UNDER WHICH THE REGULATORY REVIEW OCCURRED:

The Approved Product is a drug product, and the regulatory review of the Approved Product occurred under Section 505(b) of the Federal Food, Drug, and Cosmetic Act ("FFDCA") (21 U.S.C. § 355(b)).

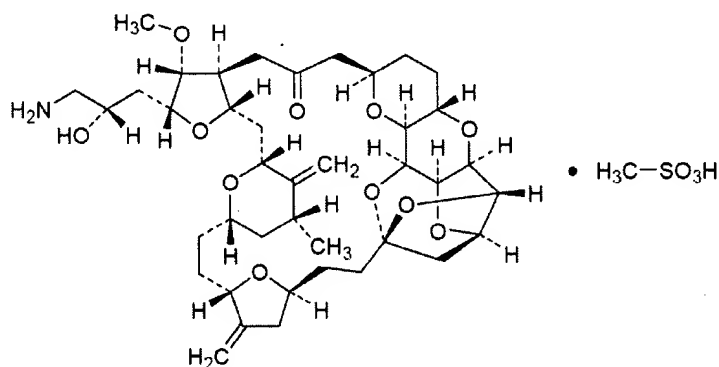
(3) AN IDENTIFICATION OF THE DATE ON WHICH THE PRODUCT RECEIVED PERMISSION FOR COMMERCIAL MARKETING OR USE UNDER THE PROVISION OF LAW UNDER WHICH THE APPLICABLE REGULATORY REVIEW PERIOD OCCURRED:

The Approved Product received permission for commercial marketing or use by FDA pursuant to Section 505(b) of the FFDCA (21 U.S.C. § 355(b)) on November 15, 2010. A copy of the approval letter is attached hereto as Exhibit 4.

(4) IN THE CASE OF A DRUG PRODUCT, AN IDENTIFICATION OF EACH ACTIVE INGREDIENT IN THE PRODUCT AND AS TO EACH ACTIVE INGREDIENT, A STATEMENT THAT IT HAS NOT BEEN PREVIOUSLY APPROVED FOR COMMERCIAL

MARKETING OR USE UNDER THE FFDCA, THE PUBLIC HEALTH SERVICE ACT, OR THE VIRUS-SERUM-TOXIN ACT, OR A STATEMENT OF WHEN THE ACTIVE INGREDIENT WAS APPROVED FOR COMMERCIAL MARKETING OR USE (EITHER ALONE OR IN COMBINATION WITH OTHER ACTIVE INGREDIENTS), THE USE FOR WHICH IT WAS APPROVED, AND THE PROVISION OF LAW UNDER WHICH IT WAS APPROVED:

FDA has approved HALAVEN™ under Section 505(b) of the FFDCA (21 U.S.C. § 355(b)) for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting. The active ingredient in HALAVEN™ is eribulin mesylate. The chemical name of eribulin mesylate is 11,15:18,21:24,28-Trieпоxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one, 2-[(2*S*)-3-amino-2-hydroxypropyl]hexacosahydro-3-methoxy-26-methyl-20,27-bis(methylene)-, (2*R*,3*R*,3*aS*,7*R*,8*aS*,9*S*,10*aR*,11*S*,12*R*,13*aR*,13*bS*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*, 29*aS*)-, methanesulfonate (salt), with the chemical structure:



Its molecular formula is $C_{40}H_{59}NO_{11} \cdot CH_4O_3S$, and its molecular weight is 826.0 (729.9 for free base).

Eribulin mesylate has not been previously approved for commercial marketing under the FFDCA, the Public Health Service Act, or the Virus-Serum-Toxin Act. In addition, no drug having the same active moiety, as defined in 21 C.F.R. § 314.108(a), as HALAVENT™ has previously been approved for commercial marketing or use under the FFDCA, the Public Health Service Act, or the Virus-Serum-Toxin Act.

(5) A STATEMENT THAT THE APPLICATION IS BEING SUBMITTED WITHIN THE SIXTY DAY PERIOD PERMITTED FOR SUBMISSION PURSUANT TO SECTION 1.720(f) AND AN IDENTIFICATION OF THE DATE OF THE LAST DAY ON WHICH THE APPLICATION COULD BE SUBMITTED:

Pursuant to 35 U.S.C. § 156(d)(1), this Application is timely filed within the sixty-day period that began on November 15, 2010 when the product received permission for commercial marketing under 21 U.S.C. § 355(b). The expiration of the sixty-day period is January 13, 2011, in accordance with 37 C.F.R. § 1.7(a). Therefore, the Application is timely filed within the sixty-day period that began on November 15, 2010 and that will expire on January 13, 2011.

(6) A COMPLETE IDENTIFICATION OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT BY THE NAME OF THE INVENTOR, THE PATENT NUMBER, THE DATE OF ISSUE, AND THE DATE OF EXPIRATION:

U.S. PATENT NO.: 6,214,865

INVENTORS: LITTLEFIELD, *ET AL.*

DATE OF ISSUE: APRIL 10, 2001

EXPIRATION DATE: JUNE 16, 2019

The expiration date of the '865 patent is June 16, 2019, based on the following: The '488 application, which was filed on June 16, 1999 and matured into the '865 patent, claims priority to Provisional Application No. 60/089,682, filed on June 17, 1998. The term is therefore twenty years from the June 16, 1999 filing date of the '488 application. Therefore, the expiration date is June 16, 2019, which is twenty years from the '488 application filing date of June 16, 1999.

(7) A COPY OF THE PATENT FOR WHICH AN EXTENSION IS BEING SOUGHT, INCLUDING THE ENTIRE SPECIFICATION (INCLUDING CLAIMS) AND DRAWINGS:

A complete copy of the '865 patent (including the specification, claims, drawings and Certificate of Correction), is attached hereto as Exhibit 1.

(8) A COPY OF ANY DISCLAIMER, CERTIFICATE OF CORRECTION, RECEIPT OF MAINTENANCE FEE PAYMENT, OR RE-EXAMINATION CERTIFICATE ISSUED IN THE PATENT:

No disclaimers were filed for the '865 patent.

The '865 patent has not been re-examined, so no re-examination certificate has issued.

A copy of the Certificate of Correction, issued on the '865 patent on January 22, 2008, is attached hereto with the complete copy of the patent, in Exhibit 1.

The first maintenance fee for the '865 patent was paid on October 7, 2004, as shown by the Patent Bibliographic Data Sheet and the USPTO Maintenance Fee Statement for this patent,

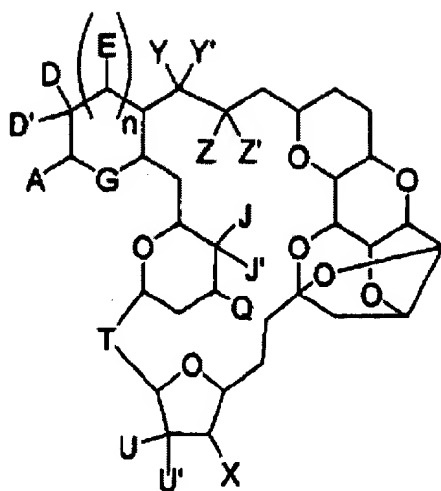
both found in Exhibit 5. The second maintenance fee for the '865 patent was paid on April 16, 2008, as shown by the Patent Bibliographic Data Sheet and the USPTO Maintenance Fee Statement for this patent. The next maintenance fee is not yet due. Accordingly, there are no unpaid maintenance fees for this patent.

(9) A STATEMENT THAT THE PATENT CLAIMS THE APPROVED PRODUCT, OR A METHOD OF USING OR MANUFACTURING THE APPROVED PRODUCT, AND A SHOWING WHICH LISTS EACH APPLICABLE PATENT CLAIM AND DEMONSTRATES THE MANNER IN WHICH AT LEAST ONE SUCH PATENT CLAIM READS ON THE APPROVED PRODUCT OR A METHOD OF USING OR MANUFACTURING THE APPROVED PRODUCT:

The '865 patent claims the Approved Product. At least claims 1, 2, 3, 5, 7, 8, 9, 10, 11, 13, 14, 15, 16, and 19 read on the Approved Product. Pursuant to 37 C.F.R. § 1.740(a)(9), a showing which demonstrates the manner in which at least one patent claim reads on the Approved Product is set forth below.

Claim 1 of the '865 patent reads as follows:

1. A compound having the formula:



wherein A is a C₁₋₆ saturated or C₂₋₆ unsaturated hydrocarbon skeleton, said skeleton being unsubstituted or having between 1 and 10 substituents, inclusive, independently selected from cyano, halo, azido, oxo, and Q₁;

each Q₁ is independently selected from OR₁, SR₁, SO₂R₁, OSO₂R₁, NR₂R₁, NR₂(CO)R₁, NR₂(CO)(CO)R₁, NR₄(CO)NR₂R₁, NR₂(CO)OR₁, (CO)OR₁, O(CO)R₁, (CO)NR₂R₁, and O(CO)NR₂ R₁;

each of R₁, R₂, R₄, R₅, and R₆ is independently selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₁₋₆ hydroxyalkyl, C₁₋₆ aminoalkyl, C₆₋₁₀ aryl, C₆₋₁₀ haloaryl, C₆₋₁₀ hydroxyaryl, C₁₋₃ alkoxy-C₆ aryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ aryl, C₆₋₁₀ haloaryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ haloaryl, (C₁₋₃ alkoxy-C₆ aryl)-C₁₋₃ alkyl, C₂₋₉ heterocyclic radical, C₂₋₉ heterocyclic radical-C₁₋₆ alkyl, C₂₋₉ heteroaryl, and C₂₋₉ heteroaryl-C₁₋₆ alkyl;

each of D and D' is independently selected from R₃ and OR₃, wherein R₃ is H, C₁₋₃ alkyl, or C₁₋₃ haloalkyl;

n is 0 or 1;

E is R₅ or OR₅;

G is O, S, CH₂, or NR₆;

each of J and J' is independently H, C₁₋₆ alkoxy, or C₁₋₆ alkyl; or J and J' taken together are =CH₂ or —O-(straight or branched C₁₋₅alkylene)-O—;

Q is C₁₋₃ alkyl;

T is ethylene or ethenylene, optionally substituted with (CO)OR₇, where R₇ is H or C₁₋₆ alkyl;

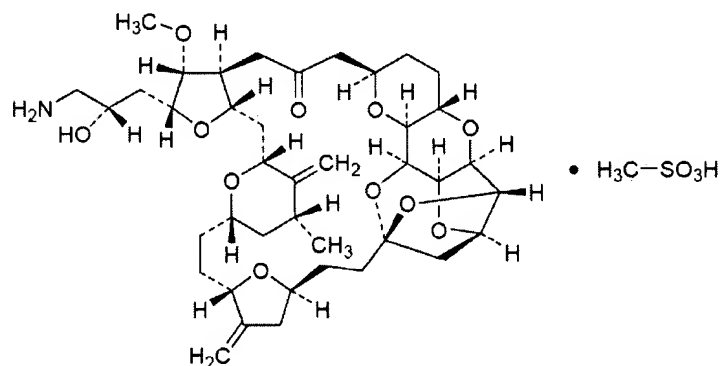
each of U and U' is independently H, C₁₋₆ alkoxy, or C₁₋₆ alkyl; or U and U' taken together are =CH₂ or —O-(straight or branched C₁₋₅ alkylene)-O—;

X is H or C₁₋₆ alkoxy;

each of Y and Y' is independently H or C₁₋₆ alkoxy; or Y and Y' taken together are =O, =CH₂, or —O-(straight or branched C₁₋₅ alkylene)-O—; and
 each of Z and Z' is independently H or C₁₋₆ alkoxy; or Z and Z' taken together are =O, =CH₂, or —O-(straight or branched C₁₋₅ alkylene)-O—;
 or a pharmaceutically acceptable salt thereof.

'865 patent, col.129 l.11–col.130 l.18 and Certificate of Correction at p. 41.

The approved labeling, attached as Exhibit 4, depicts the structure eribulin mesylate as having the following structure:



The following is a side-by-side comparison of between the structure of claim 1 of the '865 patent and the structure of HALAVEN™ (eribulin mesylate) as depicted in the Approved Labeling:

'865 Patent, claim 1	HALAVEN™ Approved Labeling

The basic structure of HALAVEN™ is the same as the basic structure of the compound of claim 1 of the '865 patent.

In HALAVEN™ (eribulin mesylate), the structure corresponding to **A** in the structure of claim 1 is a C₃ saturated hydrocarbon chain (skeleton), having two substituents (-OH and -NH₂). The -OH and -NH₂ substituents of HALAVEN™ fall with the definition of Q₁ of claim 1 of the '865 patent. The -OH substituent of HALAVEN™ meets the definition of -OR₁ of claim 1 where R₁ is H. The -NH₂ substituent meets the definition of -NR₂R₁, where both R₁ and R₂ are H.

In HALAVEN™, the structure corresponding to **D** in claim 1 of the '865 patent is -OR₃, where R₃ is C₁ alkyl. In other words, the structure in HALAVEN™ corresponding to **D** in claim 1 is a methoxy group, -OCH₃.

In HALAVEN™, the structure corresponding to **D'** in claim 1 of the '865 patent is R₃, where R₃ is -H.

In HALAVEN™, **n** is 0, meaning that the structure corresponding to **E** in claim 1 of the '865 patent is not present, as permitted by claim 1.

In HALAVEN™, the structure corresponding to **G** in claim 1 of the '865 patent is oxygen (—O—).

In HALAVEN™, the structure corresponding to **J and J'** in claim 1 of the '865 patent is =CH₂.

In HALAVEN™, the structure corresponding to **Q** in claim 1 of the '865 patent is C₁ alkyl (methyl, —CH₃).

In HALAVEN™, the structure corresponding to **T** in claim 1 of the '865 patent is ethylene.

In HALAVEN™, the structure corresponding to **U and U'** in claim 1 of the '865 patent is =CH₂.

In HALAVEN™, the structure corresponding to **X** in claim 1 of the '865 patent is hydrogen (—H).

In HALAVEN™, the structures corresponding to **Y and Y'** in claim 1 of the '865 patent are each hydrogen (—H).

In HALAVEN™, the structure corresponding to **Z and Z'** in claim 1 of the '865 patent is =O.

The Approved Product is eribulin mesylate, a pharmaceutically acceptable salt of the eribulin compound. Therefore, claim 1 reads on HALAVEN™, the Approved Product.

(10) A STATEMENT BEGINNING ON A NEW PAGE OF THE RELEVANT DATES AND INFORMATION PURSUANT TO 35 U.S.C. § 156(g) IN ORDER TO ENABLE THE SECRETARY OF HEALTH AND HUMAN SERVICES OR THE SECRETARY OF AGRICULTURE, AS APPROPRIATE, TO DETERMINE THE APPLICABLE REGULATORY REVIEW PERIOD AS FOLLOWS:

(i) FOR A PATENT CLAIMING A HUMAN DRUG, ANTIBIOTIC, OR HUMAN BIOLOGICAL PRODUCT, THE EFFECTIVE DATE OF THE INVESTIGATIONAL NEW DRUG APPLICATION (IND) AND THE IND NUMBER; THE DATE ON WHICH A NEW DRUG APPLICATION (NDA) OR A PRODUCT LICENSE APPLICATION (PLA) WAS INITIALLY SUBMITTED AND THE NDA OR PLA NUMBER; AND THE DATE ON WHICH THE NDA WAS APPROVED OR THE PRODUCT LICENSE ISSUED;

An original investigational new drug application (“IND”) was filed on March 31, 2003, and assigned IND No. 67,193. A copy of the letter acknowledging receipt of the IND is attached as Exhibit 6. Accordingly, IND No. 67,193 became effective thirty days from March 31, 2003, which is April 30, 2003.

A new drug application (“NDA”) was submitted on March 30, 2010 and acknowledged as received on March 30, 2010 (see Exhibit 7). The NDA number assigned to the application for eribulin mesylate was NDA No. 20-1532. The NDA was approved on November 15, 2010.

(11) A BRIEF DESCRIPTION BEGINNING ON A NEW PAGE OF THE SIGNIFICANT ACTIVITIES UNDERTAKEN BY THE MARKETING APPLICANT, DURING THE APPLICABLE REGULATORY REVIEW PERIOD WITH RESPECT TO THE APPROVED PRODUCT AND THE SIGNIFICANT DATES APPLICABLE TO SUCH ACTIVITIES:

In accordance with 37 C.F.R. § 1.740(a)(11), a list of significant activities, undertaken by the Marketing Applicant in each of IND No. 67,193 and NDA No. 20-1532 during the applicable regulatory review period with respect to the Approved Product is provided at: Exhibit No. 8 (IND No. 67,193) and Exhibit No. 9 (NDA No. 20-1532).

(12) A STATEMENT BEGINNING ON A NEW PAGE THAT IN THE OPINION OF THE APPLICANT THE PATENT IS ELIGIBLE FOR THE EXTENSION AND A STATEMENT AS TO THE LENGTH OF EXTENSION CLAIMED, INCLUDING HOW THE LENGTH OF THE EXTENSION WAS DETERMINED:

(a) Statement of eligibility of the patent for extension under 35 U.S.C. § 156(a):

Section 156(a) provides, in relevant part, that the term of a patent which claims a product, method of using a product, or a method of manufacturing a product shall be extended if (1) the term of the patent has not expired before an application for extension is submitted; (2) the term of the patent has never been extended under 35 U.S.C. § 156(e)(1); (3) the application for extension is submitted by the owner of record of the patent or its agent in accordance with 35 U.S.C. § 156(d); (4) the product has been subject to a regulatory review period before its commercial marketing or use; and (5) the permission for the commercial marketing or use of the product after such regulatory review is the first permitted commercial marketing or use of the product using the provision of law under which such regulatory review period occurred.

As described by corresponding number, each of these elements is satisfied:

(1) Pursuant to 35 U.S.C. § 154, the term of the '865 patent is currently set to expire on June 16, 2019. Thus, this Application is being submitted prior to the expiration of the term of the '865 patent.

(2) The term of the '865 patent has never been extended under 35 U.S.C. § 156(e)(1).

(3) This application is being submitted by Eisai R&D Management Co., Ltd., the owner of record based on duly recorded assignments discussed herein. (See Exhibit 2.) This application is submitted within the sixty-day period beginning on November 15, 2010, the date the product received permission for marketing under Section 505(b) of the FFDCA (21 U.S.C.

§ 355(b)), and ending on January 13, 2011. Additionally, the application contains the information required under 35 U.S.C. § 156(d). Therefore, it is submitted in accordance with 35 U.S.C. § 156(d).

(4) As evidenced by the letter from FDA to Eisai Inc., November 15, 2010 (Exhibit 4), the product was subject to a regulatory review period under Section 505(b) of the FFDCA (21 U.S.C. § 355(b)) before its commercial marketing or use.

(5) The permission for the commercial marketing of HALAVENT™ is the first permitted commercial marketing and use under Section 505 of the FFDCA (21 U.S.C. § 355) of the product, as defined in 35 U.S.C. § 156(f). (See section 4, above).

(b) Statement as to length of extension claimed:

The term of U.S. Patent No. 6,214,865, now expiring on June 16, 2019, should be extended for 1,495 days, or to July 20, 2023, in accordance with 35 U.S.C. § 156.

As set forth in 35 U.S.C. § 156(g)(1), the regulatory review period equals the length of time between the effective date of IND No. 67,193 of April 30, 2003 and the submission of NDA No. 20-1532 on March 30, 2010, a period of 2,527 days (i.e., the “testing phase”), plus the length of time between the submission of the NDA No. 20-1532 on March 30, 2010 to NDA approval on November 15, 2010 (i.e., the “approval phase”), a period of 231 days. These two periods added together equal 2,758 days.

Pursuant to 37 C.F.R. § 1.775(d), the term of the patent is extended and determined by subtracting from the 2,758 day regulatory review period the following:

(i) 0 days, which is the number of days in the IND and NDA periods on or before the issuance of U.S. Patent No. 6,214,865 on April 10, 2001.

(ii) 1,263 days, which is one half the number of days remaining in the IND period after the subtraction of 0 days above (wherein half days are ignored for purposes of this subtraction, as provided by 37 C.F.R. § 1.775(d)(1)(iii)).

From the foregoing calculation, an extension of 1,495 days results, i.e., the remaining period under 35 U.S.C. § 156(g)(1)(B)(i) (1,264 days) plus the remaining period under 35 U.S.C. § 156(g)(1)(B)(iii) (231 days), or 1,495 days. This length of an extension would provide a new expiration date for U.S. Patent No. 6,214,865 of July 20, 2023. However, this extension period is subject to two further potential limitations under 35 U.S.C. § 156.

First, under 35 U.S.C. § 156(g)(6)(A), a maximum extension of five years is permitted. In this case, since the current expiry date of U.S. Patent No. 6,214,865 is June 16, 2019, no patent term extension could extend the term of the patent beyond June 16, 2024. Consequently, this provision does not operate to limit the possible extension available to U.S. Patent No. 6,214,865.

Second, under 35 U.S.C. § 156(c)(3), if the calculated extension period would result in a patent term extending beyond fourteen years after the date of FDA approval for HALAVENT™, that is, a patent term expiring after November 15, 2024, the period of extension would be limited so that this period does not exceed fourteen years. In this case, that provision also does not operate to limit the possible extension available to U.S. Patent No. 6,214,865.

Accordingly, U.S. Patent No. 6,214,865 is eligible for a patent term extension of 1,495 days.

(13) A STATEMENT THAT THE APPLICANT ACKNOWLEDGES A DUTY TO DISCLOSE TO THE DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE AND THE SECRETARY OF HEALTH AND HUMAN SERVICES OR THE SECRETARY OF AGRICULTURE ANY INFORMATION WHICH IS MATERIAL TO THE DETERMINATION OF ENTITLEMENT TO THE EXTENSION SOUGHT (SEE 37 C.F.R. § 1.765).

Eisai R&D Management Co., Ltd., the Applicant, acknowledges a duty to disclose to the Director of the United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

(14) THE PRESCRIBED FEE FOR RECEIVING AND ACTING UPON THE APPLICATION FOR EXTENSION (SEE 37 C.F.R. § 1.20(j)):

The Director is hereby authorized to charge our Deposit Account 50-0740 in the amount of \$1,120.00 to cover the fee for this application for extension of patent term. The Director is also hereby authorized to charge our Deposit Account 50-0740, for any deficiency in the fees filed, asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm), to prevent this application from being inadvertently abandoned. A duplicate of this Request (without Exhibits 1-9) is attached.

(15) THE NAME, ADDRESS, AND TELEPHONE NUMBER OF THE PERSON TO WHOM INQUIRIES AND CORRESPONDENCE RELATING TO THE APPLICATION FOR PATENT TERM EXTENSION ARE TO BE DIRECTED:

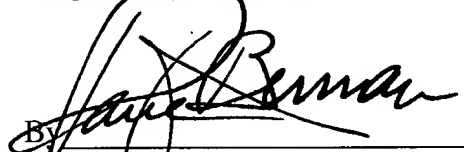
Paul J. Berman
Christopher N. Sipes
COVINGTON & BURLING LLP
1201 Pennsylvania Avenue, N.W.
Washington, D.C. 20004-2401
Telephone No.: 202.662.6000
Facsimile No.: 202.662.6291

Pursuant to 37 C.F.R. § 1.740(b), this Application for Extension of patent Term under 35 U.S.C. § 156 includes Exhibits 1–9, and is accompanied by two additional copies. Pursuant to M.P.E.P. § 2753, an additional two copies of this Request, with accompanying Exhibits 1–9, also accompanies this Application.

Accordingly, submitted herewith are a total of: one original Application including Exhibits; four copies of this Application including Exhibits; and one copy of this Application without Exhibits.

Dated: January 11, 2011

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Paul J. Berman", is written over a horizontal line.

Paul J. Berman
Registration No.: 36,744
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PATENT – POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS		Patent Number		6,214,865		
		Issue Date		April 10, 2001		
		First Named Inventor		Bruce A. Littlefield		
		Title	MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND			
		Attorney Docket No.		029163.0022-US01		

I hereby revoke all previous powers of attorney given in the above-identified patent.

☐ A Power of Attorney is submitted herewith.
OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

26853

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) with respect to the patent identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number	Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified patent to:

☒ The address associated with the above-mentioned Customer Number.
OR

☐ The address associated with Customer Number:

26853

OR

☐ Firm or Individual Name

Address

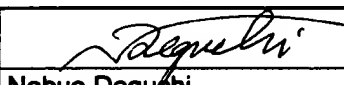
City	State	Zip	
Country	Telephone	Email	

I am the:

☐ Inventor, having ownership of the patent.
OR

☒ Patent owner.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on (submitted herewith)

SIGNATURE of Inventor or Patent Owner

Signature 	Date December 29, 2010
Name Nobuo Deguchi	Telephone +81-3-3817-5190
Title and Company President — Eisai R&D Management Co., Ltd.	

NOTE: Signatures of all the inventors or patent owners of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below.

☒ *Total of 1 forms are submitted.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)Applicant/Patent Owner: Bruce A. Littlefield, et al., / Eisai R&D Management Co., Ltd.Application No./Patent No.: 09/334,488 / 6,214,865 Filed/Issue Date: June 16, 1999 / April 10, 2001Titled: MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATIONEisai R&D Management Co., Ltd., a corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest in;
2. ☐ an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or
3. ☐ an assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)
the patent application/patent identified above by virtue of either:

- A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

OR

- B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Bruce A. Littlefield, et al., To: Eisai Co., Ltd.
The document was recorded in the United States Patent and Trademark Office at Reel 010118, Frame 0278, or for which a copy thereof is attached.
2. From: Eisai Co., Ltd. To: Eisai R&D Management Co., Ltd.
The document was recorded in the United States Patent and Trademark Office at Reel 020352, Frame 0458, or for which a copy thereof is attached.
3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

- ☒ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.


Signature

Nobuo Deguchi
Printed or Typed Name

December 29, 2010
Date

President
Title

EXHIBIT 1



US006214865B1

(12) **United States Patent**
Littlefield et al.

(10) **Patent No.:** **US 6,214,865 B1**
 (45) **Date of Patent:** ***Apr. 10, 2001**

(54) **MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATION**

(75) **Inventors:** **Bruce A. Littlefield**, Andover, MA (US); **Monica H. Palme**, San Jose, CA (US); **Boris M. Seletsky**, Andover, MA (US); **Murray J. Towle**, Auburn, NH (US); **Melvin J. Yu**, Andover, MA (US); **Wanjun Zheng**, Londonberry, NH (US)

(73) **Assignee:** **Elsal Co., Ltd.**, Tokyo (JP)

(*) **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **09/334,488**

(22) **Filed:** **Jun. 16, 1999**

Related U.S. Application Data

(60) Provisional application No. 60/089,682, filed on Jun. 17, 1998.

(51) **Int. Cl.⁷** **A61K 31/335; C07D 321/02**

(52) **U.S. Cl.** **514/450; 514/337; 514/338; 514/410; 514/432; 514/443; 548/417; 549/24; 549/41; 549/348; 546/281.7; 546/282.7**

(58) **Field of Search** **549/348, 24, 41; 546/281.7, 282.7; 548/417; 514/337, 338, 410, 432, 443, 453, 460, 450**

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,338,865	8/1994	Kishi et al.	549/214
5,436,238	7/1995	Kishi et al.	514/214

FOREIGN PATENT DOCUMENTS

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Horita et al., "Synthetic Studies of Halichondrin B, an Antitumor Polyether Macrolide Isolated from a Marine Sponge. 8. Synthesis of the Lactone Part (C1-C36) via Horner-Emmons Coupling Between C1-C15 and C16-C36 Fragments and Yamaguchi Lactonization," Tetrahedron Letters 38:8965-8968 (1997).

Stamos et al., "New Synthetic Route to the C.14-C.38 Segment of Halichondrins," J. Org. Chem. 62:7552-7553 (1997).

Primary Examiner—Amelia Owens

(74) *Attorney, Agent, or Firm*—Clark & Elbing LLP

(57)

ABSTRACT

The invention provides halichondrin analogs having pharmaceutical activity, such as anticancer or antimetabolic (mitosis-blocking) activity, and methods of identifying agents that induce a sustained mitotic block in a cell after transient exposure of the cell to the agents.

21 Claims, No Drawings

1

MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATION

This application claims benefit of Provisional Application Serial No. 60/089,682 filed Jun. 17, 1998.

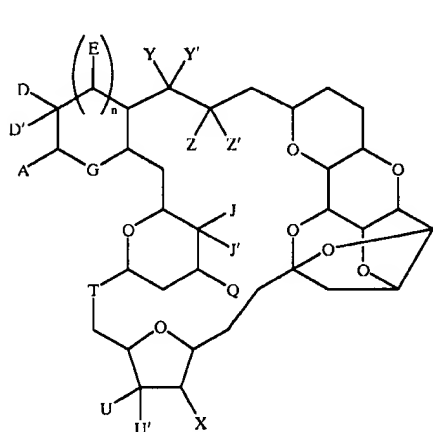
BACKGROUND

The invention relates to pharmaceutically active macrolides. Halichondrin B is a potent anticancer agent originally isolated from the marine sponge *Halichondria okadai*, and subsequently found in *Axinella* sp., *Phakellia carteri*, and *Lissonodendryx* sp.

A total synthesis of Halichondrin B was published in 1992 (Aicher, T. D. et al., *J. Am. Chem. Soc.* 114:3162-3164). Halichondrin B has demonstrated in vitro inhibition of tubulin polymerization, microtubule assembly, beta⁵-tubulin crosslinking, GTP and vinblastine binding to tubulin, and tubulin-dependent GTP hydrolysis and has shown in vitro and in vivo anti-cancer properties.

SUMMARY OF THE INVENTION

The invention provides halichondrin analogs having pharmaceutical activity, such as anticancer or antimitotic (mitosis-blocking) activity. These compounds are substantially smaller than halichondrin B. The invention features a compound having the formula (I):



Formula (I)

In formula (I), A is a C₁₋₆ saturated or C₂₋₆ unsaturated hydrocarbon skeleton, the skeleton being unsubstituted or having between 1 and 13 substituents, preferably between 1 and 10 substituents, e.g., at least one substituent selected from cyano, halo, azido, Q₁, and oxo. Each Q₁ is independently selected from OR₁, SR₁, SO₂R₁, OSO₂R₁, NR₂R₁, NR₂(CO)R₁, NR₂(CO)(CO)R₁, NR₄(CO)NR₂R₁, NR₂(CO)OR₁, (CO)OR₁, O(CO)R₁, (CO)NR₂R₁, and O(CO)NR₂R₁. The number of substituents can be, for example, between 1 and 6, 1 and 8, 2 and 5, or 1 and 4. Throughout the disclosure, numerical ranges are understood to be inclusive.

Each of R₁, R₂, R₄, R₅, and R₆ is independently selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₁₋₆ hydroxyalkyl, C₁₋₆ aminoalkyl, C₆₋₁₀ aryl, C₆₋₁₀ haloaryl (e.g., p-fluorophenyl or p-chlorophenyl), C₆₋₁₀ hydroxyaryl, C₁₋₄ alkoxy-C₆ aryl (e.g., p-methoxyphenyl, 3,4,5-trimethoxyphenyl, p-ethoxyphenyl, or 3,5-diethoxyphenyl), C₆₋₁₀ aryl-C₁₋₆ alkyl (e.g., benzyl or phenethyl), C₁₋₆ alkyl-C₆₋₁₀ aryl, C₆₋₁₀ haloaryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ haloaryl, (C₁₋₃ alkoxy-C₆aryl)-C₁₋₃ alkyl, C₂₋₉heterocyclic radical,

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C₂₋₉heterocyclic radical-C₁₋₆ alkyl, C₂₋₉heteroaryl, and C₂₋₉heteroaryl-C₁₋₆ alkyl. There may be more than one R₁, for example, if A is substituted with two different alkoxy (OR₁) groups such as butoxy and 2-aminoethoxy.

Examples of A include 2,3-dihydroxypropyl, 2-hydroxyethyl, 3-hydroxy-4-perfluorobutyl, 2,4,5-trihydroxypentyl, 3-amino-2-hydroxypropyl, 1,2-dihydroxyethyl, 2,3-dihydroxy-4-perfluorobutyl, 3-cyano-2-hydroxypropyl, 2-amino-1-hydroxy ethyl, 3-azido-2-hydroxypropyl, 3,3-difluoro-2,4-dihydroxybutyl, 2,4-dihydroxybutyl, 2-hydroxy-2(p-fluorophenyl)-ethyl, —CH₂(CO)(substituted or unsubstituted aryl), —CH₂(CO)(alkyl or substituted alkyl, such as haloalkyl or hydroxyalkyl) and 3,3-difluoro-2-hydroxypent-4-enyl.

Examples of Q₁ include —NH(CO)(CO)-(heterocyclic radical or heteroaryl), —OSO₂-(aryl or substituted aryl), —O(CO)NH-(aryl or substituted aryl), aminoalkyl, hydroxyalkyl, —NH(CO)(CO)-(aryl or substituted aryl), —NH(CO)(alkyl)(heteroaryl or heterocyclic radical), O(substituted or unsubstituted alkyl)(substituted or unsubstituted aryl), and —NH(CO)(alkyl)(aryl or substituted aryl).

Each of D and D' is independently selected from R₃ and OR₃, wherein R₃ is H, C₁₋₃ alkyl, or C₁₋₃ haloalkyl. Examples of D and D' are methoxy, methyl, ethoxy, and ethyl. In some embodiments, one of D and D' is H.

The value for n is 1 or preferably 0, thereby forming either a six-membered or five-membered ring. This ring can be unsubstituted or substituted, e.g., where E is R₅ or OR₅, and can be a heterocyclic radical or a cycloalkyl, e.g. where G is S, CH₂, NR₆, or preferably O.

Each of J and J' is independently H, C₁₋₆ alkoxy, or C₁₋₆ alkyl; or J and J' taken together are =CH₂ or —O-(straight or branched C₁₋₅alkylene or alkylidene)—O—, such as exocyclic methylenide, isopropylidene, methylene, or ethylene. Q is C₁₋₃ alkyl, and is preferably methyl. T is ethylene or ethynylene, optionally substituted with (CO)OR₇, where R₇ is H or C₁₋₆ alkyl. Each of U and U' is independently H, C₁₋₆ alkoxy, or C₁₋₆ alkyl; or U and U' taken together are =CH₂ or —O-(straight or branched C₁₋₅alkylene or alkylidene)—O—. X is H or C₁₋₆ alkoxy. Each of Y and Y' is independently H or C₁₋₆ alkoxy; or Y and Y' taken together are =O, =CH₂, or —O-(straight or branched C₁₋₅alkylene or alkylidene)—O—. Each of Z and Z' is independently H or C₁₋₆ alkoxy; or Z and Z' taken together are =O, =CH₂, or —O-(straight or branched C₁₋₅alkylene or alkylidene)—O—.

The invention features compounds of sufficient stability to be suitable for pharmaceutical development. The invention also features pharmaceutically acceptable salts of disclosed compounds, disclosed novel synthetic intermediates, pharmaceutical compositions containing one or more disclosed compounds, methods of making the disclosed compounds or intermediates, and methods of using the disclosed compounds or compositions. Methods of use include methods for reversibly or irreversibly inhibiting mitosis in a cell, and for inhibiting cancer or tumor growth in vitro, in vivo, or in a patient. The invention also features methods for identifying an anti-mitotic or anti-cancer agent, such as a reversible or, preferably, an irreversible agent.

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

B. Halichondrin Analogs

C. Synthesis of Halichondrin Analogs

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D. Pharmacological Activity

E. Uses

A. Definitions

The following terms are defined in part below and by their usage herein.

Hydrocarbon skeletons contain carbon and hydrogen atoms and may be linear, branched, or cyclic. Unsaturated hydrocarbons include one, two, three or more C—C double bonds (sp^2) or C—C triple bonds (sp). Examples of unsaturated hydrocarbon radicals include ethynyl, 2-propynyl, 1-propenyl, 2-butenyl, 1,3-butadienyl, 2-pentenyl, vinyl (ethenyl), allyl, and isopropenyl. Examples of bivalent unsaturated hydrocarbon radicals include alkenylenes and alkylidenes such as methylidyne, ethylidene, ethylidyne, vinylidene, and isopropylidene. In general, compounds of the invention have hydrocarbon skeletons ("A" in formula (I)) that are substituted, e.g., with hydroxy, amino, cyano, azido, heteroaryl, aryl, and other moieties described herein. Hydrocarbon skeletons may have two geminal hydrogen atoms replaced with oxo, a bivalent carbonyl oxygen atom ($=O$), or a ring-forming substituent, such as $-O-$ (straight or branched alkylene or alkylidene)- $O-$ to form an acetal or ketal.

C_{1-6} alkyl includes linear, branched, and cyclic hydrocarbons, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, sec-pentyl, neo-pentyl, tert-pentyl, cyclopentyl, hexyl, isohexyl, sec-hexyl, cyclohexyl, 2-methylpentyl, tert-hexyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1,3-dimethylbutyl, and 2,3-dimethyl but-2-yl. Alkoxy ($-OR$), alkylthio ($-SR$), and other alkyl-derived moieties (substituted, unsaturated, or bivalent) are analogous to alkyl groups (R). Alkyl groups, and alkyl-derived groups such as the representative alkoxy, haloalkyl, hydroxyalkyl, alkenyl, alkylidene, and alkylene groups, can be C_{2-6} , C_{3-6} , C_{1-3} , or C_{2-4} .

Alkyls substituted with halo, hydroxy, amino, cyano, azido, and so on can have 1, 2, 3, 4, 5 or more substituents, which are independently selected (may or may not be the same) and may or may not be on the same carbon atom. For example, haloalkyls are alkyl groups with at least one substituent selected from fluoro, chloro, bromo, and iodo. Haloalkyls may have two or more halo substituents which may or may not be the same halogen and may or may not be on the same carbon atom. Examples include chloromethyl, periodomethyl, 3,3-dichloropropyl, 1,3-difluorobutyl, and 1-bromo-2-chloropropyl.

Heterocyclic radicals and heteroaryls include furyl, pyranlyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathieryl, 2H-pyrrolyl, pyrrolyl, imidazolyl (e.g., 1-, 2- or 4-imidazolyl), pyrazolyl, isothiazolyl, isoxazolyl, pyridyl (e.g., 1-, 2-, or 3-pyridyl), pyrazinyl, pyrimidinyl, pyridazinyl, indolizynyl, isoindolyl, 3H-indolyl, indolyl (e.g., 1-, 2-, or 3-indolyl), indazolyl, purinyl, 4H-quinolixynyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalynyl, quinazolinyl, cinnolynyl, pteridinyl, pyrrolinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, isoindolinyl, and morpholinyl. Heterocyclic radicals and heteroaryls may be linked to the rest of the molecule at any position along the ring. Heterocyclic radicals and heteroaryls can be C_{2-9} , or smaller, such as C_{3-6} , C_{2-5} , or C_{3-7} .

Aryl groups include phenyl, benzyl, naphthyl, tolyl, mesityl, xylyl, and cumenyl.

It is understood that "heterocyclic radical", "aryl", and "heteroaryl" include those having 1, 2, 3, 4, or more substituents independently selected from lower alkyl, lower

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alkoxy, amino, halo, cyano, nitro, azido, and hydroxyl. Heterocyclic radicals, heteroaryls, and aryls may also be bivalent substituents of hydrocarbon skeleton "A" in formula (I).

B. Halichondrin Analogs

Referring to formula (I) in the Summary section, embodiments of the invention include compounds wherein n is 0; wherein each of D and D' is independently selected from R_3 , C_{1-3} alkoxy, and C_{1-3} haloalkoxy; wherein R_5 is selected from H, C_{2-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} aminoalkyl, C_{6-10} aryl, C_{6-10} haloaryl, C_{6-10} hydroxyaryl, C_{1-3} alkoxy- C_6 aryl, C_{6-10} aryl- C_{1-6} alkyl, C_{1-6} alkyl- C_{6-10} aryl, C_{6-10} haloaryl- C_{1-6} alkyl, C_{1-6} alkyl- C_{6-10} haloaryl, (C_{1-3} alkoxy- C_6 aryl)- C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heterocyclic radical- C_{1-6} alkyl, C_{2-9} heteroaryl, and C_{2-9} heteroaryl- C_{1-6} alkyl; and combinations thereof.

Other embodiment includes compounds having one or more of the following characteristics: (a) wherein A is a C_{1-6} saturated or C_{2-6} unsaturated hydrocarbon skeleton, the skeleton having at least one substituent selected from cyano, halo, azido, Q_1 , and oxo; (b) each Q_1 is independently selected from OR_1 , SR_1 , SO_2R_1 , OSO_2R_1 , NR_2R_1 , $NR_2(CO)R_1$, $NR_2(CO)R_1$, and $O(CO)NR_2R_1$; (c) Z and Z' taken together are $=O$ or $=CH_2$; (d) wherein each Q_1 is independently selected from OR_1 , SR_1 , SO_2R_1 , OSO_2R_1 , $NH(CO)R_1$, $NH(CO)(CO)R_1$, and $O(CO)NHR_1$; (e) each R_1 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_6 aryl, C_6 haloaryl, C_{1-3} alkoxy- C_6 aryl, C_6 aryl- C_{1-3} alkyl, C_{1-3} alkyl- C_6 aryl, C_6 haloaryl- C_{1-3} alkyl, C_{1-3} alkyl- C_6 haloaryl, (C_{1-3} alkoxy- C_6 aryl)- C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heteroaryl, and C_{2-9} heteroaryl- C_{1-6} alkyl; (f) one of D and D' is methyl or methoxy and the other is H; (g) n is 0; (h) G is O; (i) J and J' taken together are $=CH_2$; (j) Q is methyl; (k) T is ethylene; (l) U and U' taken together are $=CH_2$; (m) X is H; (n) each of Y and Y' is H; and (o) Z and Z' taken together are $=O$. Examples of combinations are the combination of (h)-(m), the combination of (a) and (b), the combination of (f) and (h), and the combination of (h) and where one of D and D' is methyl and the other is H. Two particularly preferred compounds are B1793 and B13939.

Another embodiment includes compounds wherein Q_1 is independently selected from OR_1 , SR_1 , SO_2R_1 , and OSO_2R_1 ; and each R_1 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_6 aryl, C_6 haloaryl, C_{1-3} alkoxy- C_6 aryl, C_6 aryl- C_{1-3} alkyl, C_{1-3} alkyl- C_6 aryl, C_6 haloaryl- C_{1-3} alkyl, C_{1-3} alkyl- C_6 haloaryl, and (C_{1-3} alkoxy- C_6 aryl)- C_{1-3} alkyl. Other embodiments include compounds wherein: one of D and D' is alkyl or alkoxy, where n is 1; (f) as above, where n is 1; E is alkoxy, where n is 1; n is 0, where one of D and D' is hydroxy and the other is H; and (f) as above, where n is 1 and E is methyl.

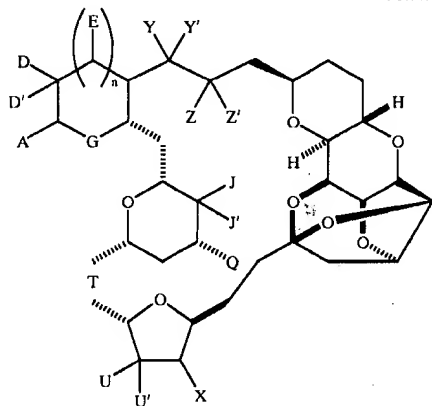
The invention also features compounds wherein: (1) A has at least one substituent selected from hydroxyl, amino, azido, halo, and oxo; (2) A is a saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl, amino, and azido (e.g., B1793, B13939, B2042, B1794, and B1922); (3) A has at least two substituents independently selected from hydroxyl, amino, and azido (e.g., B2090 and B2136); (4) A has at least two substituents independently selected from hydroxyl and amino (e.g., B2042 and B2090); (5) A has at least one hydroxyl substituent and at least one amino substituent (e.g., B1939 and B2136); (6) A has at least two hydroxyl substituents (e.g., B1793 and B1794); (7) A is a C_{2-4} hydrocarbon skeleton that is substituted (e.g., B2004, B2037, B1920, B2039, B2070, B2090, and B2043); (8) A is a C_3 hydrocarbon skeleton that

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is substituted (e.g., B1793, B1920, B1984, B1988, B1939, B1940, B2014); (9) A has an (S)-hydroxyl alpha to the carbon atom linking A to the ring containing G (e.g., B1793, B1939 or B1920) or an (R)-hydroxyl (e.g., B2102, B2013, B2042); and (10) A is a C₁₋₆ saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl and cyano (e.g., B2013, B2037, B2102, B2086, and B2091). By (S)-hydroxyl is meant the configuration of the carbon atom having the hydroxyl group is (S). Embodiments of the invention also include compounds which have at least two substituents on the carbon atoms (1) alpha and gamma, (2) beta and gamma, or preferably (3) alpha and beta to the carbon atom linking A to the ring containing G. The alpha, beta, and gamma carbon atoms can have an (R) or (S) configuration

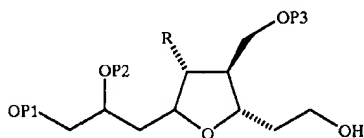
The invention further provides preferred compounds having the formula (I) -A, shown below, wherein the substituents are identical to those defined above.

Formula 1-A



The invention further features the following monosaccharide intermediate having formula (II):

Formula (II)



wherein R is methyl or methoxy, and each of P1, P2, and P3 is independently selected from H and primary alcohol protecting groups. Preferably, the diol sidechain is below the plane of the page and OP2 is above the plane of the page. Primary alcohol protecting groups include esters, ethers, silyl ethers, alkyl ethers, and alkoxyalkyl ethers.

Examples of esters include formates, acetates, carbonates, and sulfonates. Specific examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio) pentanoate, pivaloate, crotonate, 4-methoxy-crotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl) ethyl, vinyl, allyl, and p-nitrobenzyl.

Examples of silyl ethers include trimethylsilyl, triethylsilyl, t-butyltrimethylsilyl, t-butylphenylsilyl,

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triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyranyl ethers. Examples of benzyl ethers include p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picolyl. Preferably, each of P1 and P2 are TBS and P3 is MPM (see alcohol 19 below). In one aspect, formula (II) can be modified so the hydroxyethyl sidechain can also be a protected hydroxyl, —CH₂CH₂O—P4, wherein P4 is independently selected from values for P1. A related intermediate is alcohol 17, where the hydroxyethyl sidechain is a hydroxymethyl sidechain. A corresponding hydroxypropyl sidechain, or aminoalkyl side chain, can be similarly prepared.

P1 and P2, taken together, can be a diol protecting group, such as cyclic acetals and ketals (methylene, ethylidene, benzylidenes, isopropylidene, cyclohexylidene, and cyclopentylidene), silylene derivatives such as di-t-butylsilylene and 1,1,3,3-tetra-isopropylidene derivatives, cyclic carbonates, and cyclic boronates. Methods of adding and removing such hydroxyl protecting groups, and additional protecting groups, are well-known in the art and available, for example, in P. J. Kocienski, *Protecting Groups*, Thieme, 1994, and in T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 2nd edition, John Wiley & Sons, 1992.

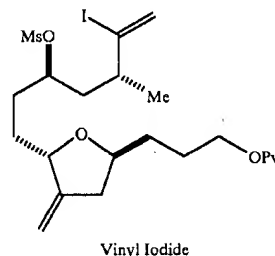
The following section provides representative syntheses of intermediates of formula (II) and halichondrin analogs of formula (I).

C. Synthesis of Halichondrin Analogs

An overview is provided below, followed by synthetic schemes 1-16, and several detailed protocols.

Compounds of general formula 4 can be prepared by the route outlined in Scheme 1. Key fragment F-2 exemplified by vinyl iodide compound X2 can be prepared according to the procedure of Kishi, et al. (Total synthesis of halichondrin B and norhalichondrin B. Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* 1992, 114, 3162-4).

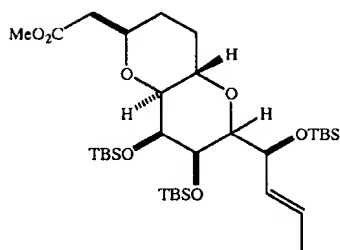
X2



Vinyl Iodide

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-continued



XF3

Key fragment F-3 can be obtained by DIBALH reduction of the corresponding methyl ester, XF3, prepared according to the procedure of Stamos, et al (Scheme 2). [Synthetic studies on halichondrins: a practical synthesis of the C.1-C.13 segment. Stamos, D. P.; Kishi, Y. *Tetrahedron Lett.* 1996, 37, 8643-8646]. Synthesis of key fragment F-1 exemplified by compound 20 can be synthesized as described in Scheme 3 or Scheme 4.

Using B1793 as a representative example, coupling of the three key fragments proceeded as outlined in Scheme 5: Nozaki-Hiyama-Kishi coupling of fragments 20 and X2 followed by intramolecular Williamson ether formation furnished tetrahydropyran B2318. Protecting group modification as described in Scheme 5 or alternatively in Scheme 6 afforded primary iodide B2313. Halogen-metal exchange reaction and coupling with key fragment F-3 furnished a mixture of diastereomeric alcohols B2308. Additional protecting group manipulation and oxidation followed by an intramolecular Nozaki-Hiyama-Kishi reaction afforded an intermediate, which when oxidized and treated with TBAF underwent intramolecular hetero-Michael ring closure. PPIs mediated acetal formation furnished B1793.

Aryl groups can be incorporated into the C32 sidechain (e.g. B2043) as exemplified in Scheme 7. Intermediate B2318 was deprotected and the resulting diol oxidatively cleaved to the corresponding aldehyde. Treatment with a Grignard reagent (e.g. p-F-PhMgBr), separation of the resulting diastereomers and silylation furnished 204, which was converted to final product in a manner similar to that described in Scheme 6.

Ether analogs can be prepared from B1793 by treatment with an appropriate alkylating agent (e.g. Scheme 8). Similarly, sulfonates, esters, carbamates, etc. can be prepared from B1793 by treatment with an activated carbonyl component. Oxidative diol cleavage and reduction or selective hydroxyl group oxidation could furnish derivatives such as B2037 and B1934, respectively.

Alternatively, one or more hydroxyl groups could be converted to the corresponding amino groups with subsequent coupling with an activated carbonyl component (Scheme 9). Displacement of the sulfonyl intermediate (e.g. B1920) by carbon or heteroatom nucleophiles could also be readily accomplished (Scheme 10).

C31 methyl analogs can be prepared as outlined in Scheme 11. Indium mediated coupling of an allyl bromide ester with 2,3-O-(3-isopropylidene)-D-glyceraldehyde furnished lactone 103. Hetero Michael addition, lactone reduction, Wittig coupling and intramolecular Michael addition furnished tetrahydrofuran 107. Pummerer

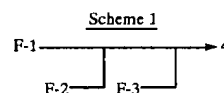
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rearrangement, protecting group adjustment and DIBALH reduction furnished key fragment 1 (e.g., 114), which was converted to final compound in a manner analogous to that described in Scheme 6.

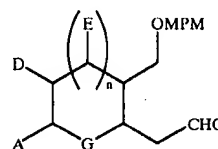
Fluorine atoms could be introduced as described in Schemes 12-14. Beginning with the appropriate tetrahydrofuran intermediate, fluorinated key fragment 1 was obtained and carried to final compound in a manner analogous to that illustrated in Scheme 6.

Triol derivatives could be similarly prepared from the tetrahydrofuran intermediate. For example, as outlined in Scheme 15 allyltributylstannane addition to aldehyde X32 furnished homoallylic alcohol 33 that was carried to final compound in a similar manner to that described in Scheme 6. These triols could be further modified as exemplified in Scheme 6.

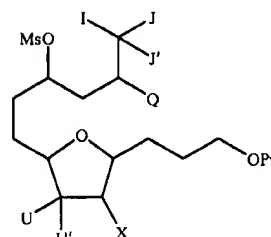
The 1,3 diol derivatives could be prepared from intermediates previously described. For example, B2086 could be oxidatively cleaved and reduced to afford 1,3-diol B2091 (Scheme 16).



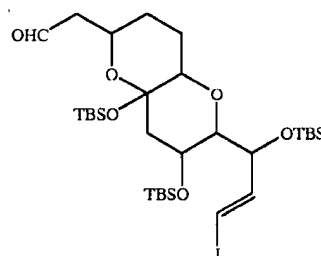
F-1



F-2



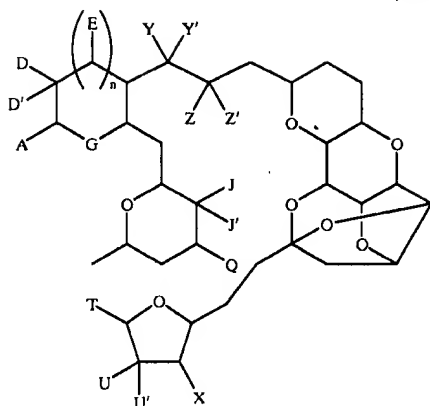
F-3



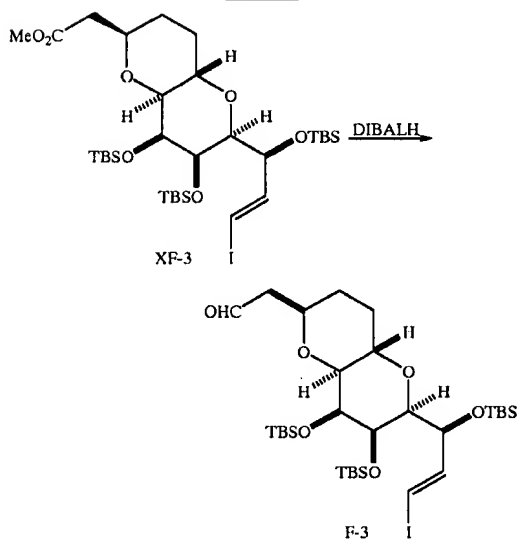
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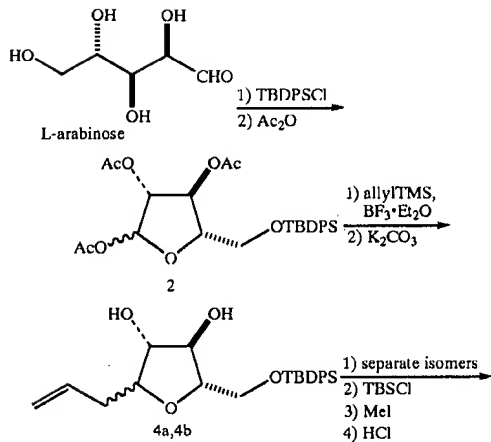
4, Formula (I)



Scheme 2

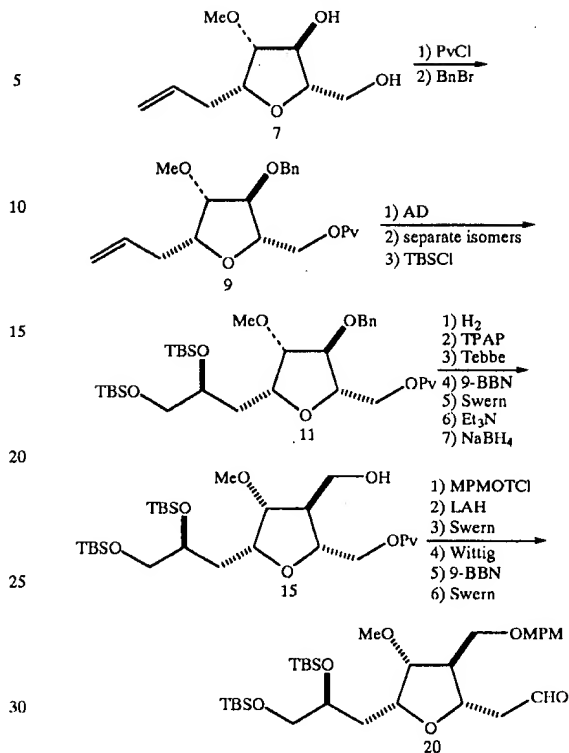


Scheme 3

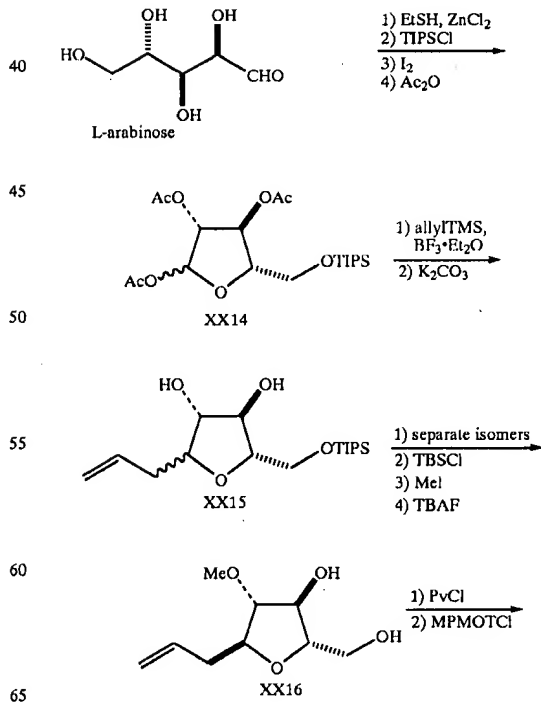


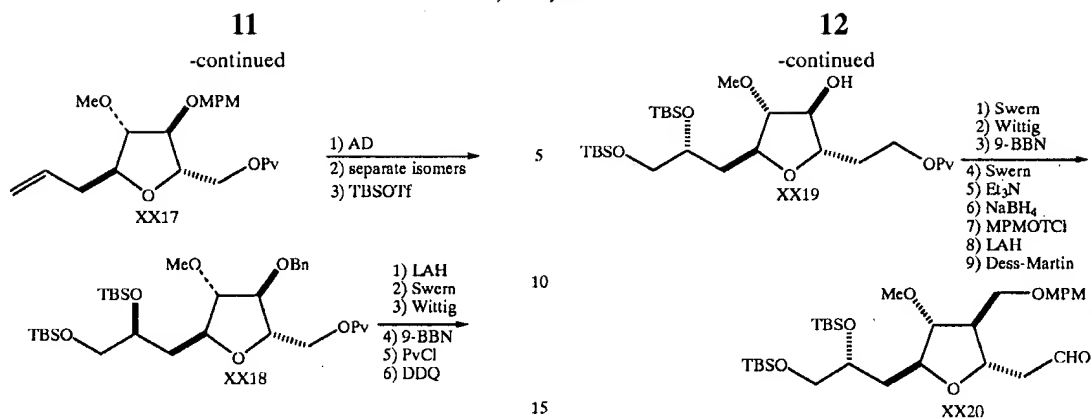
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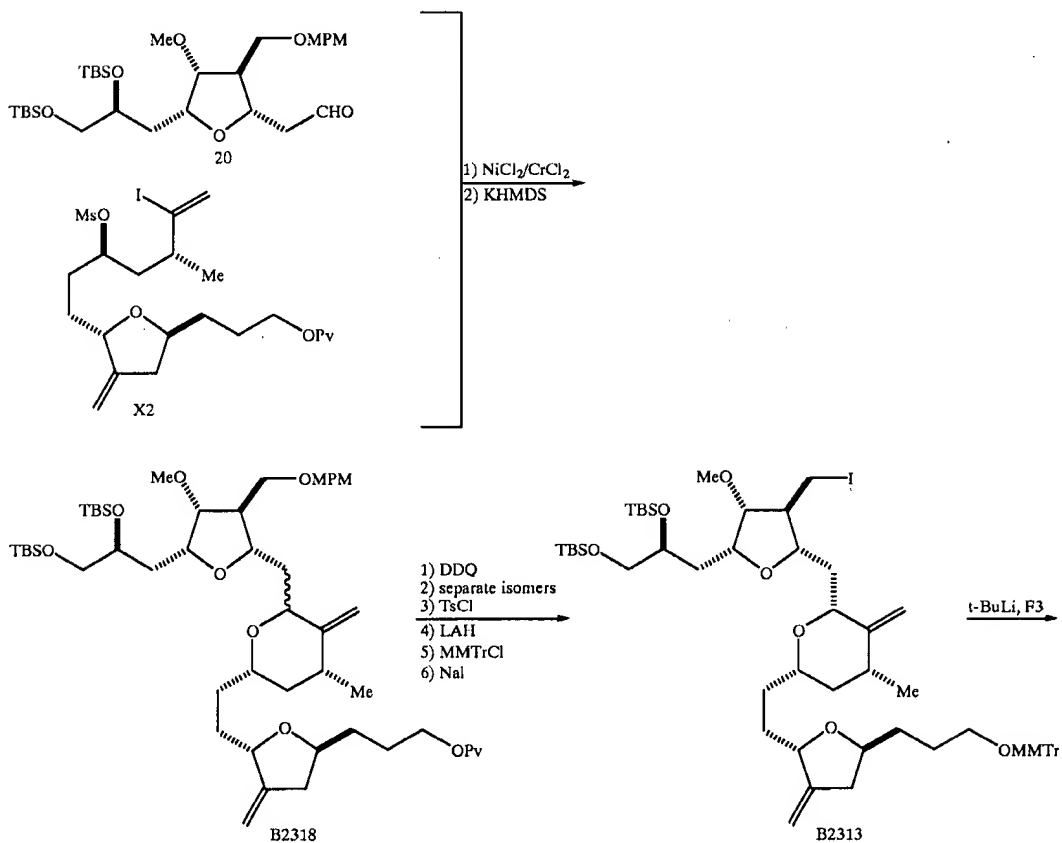


Scheme 4





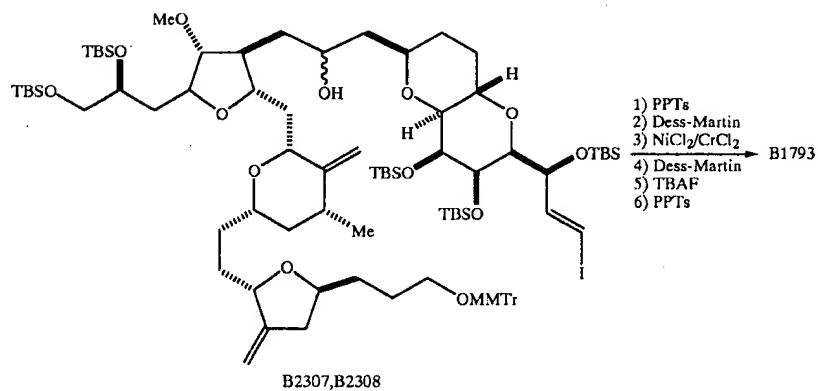
Scheme 5



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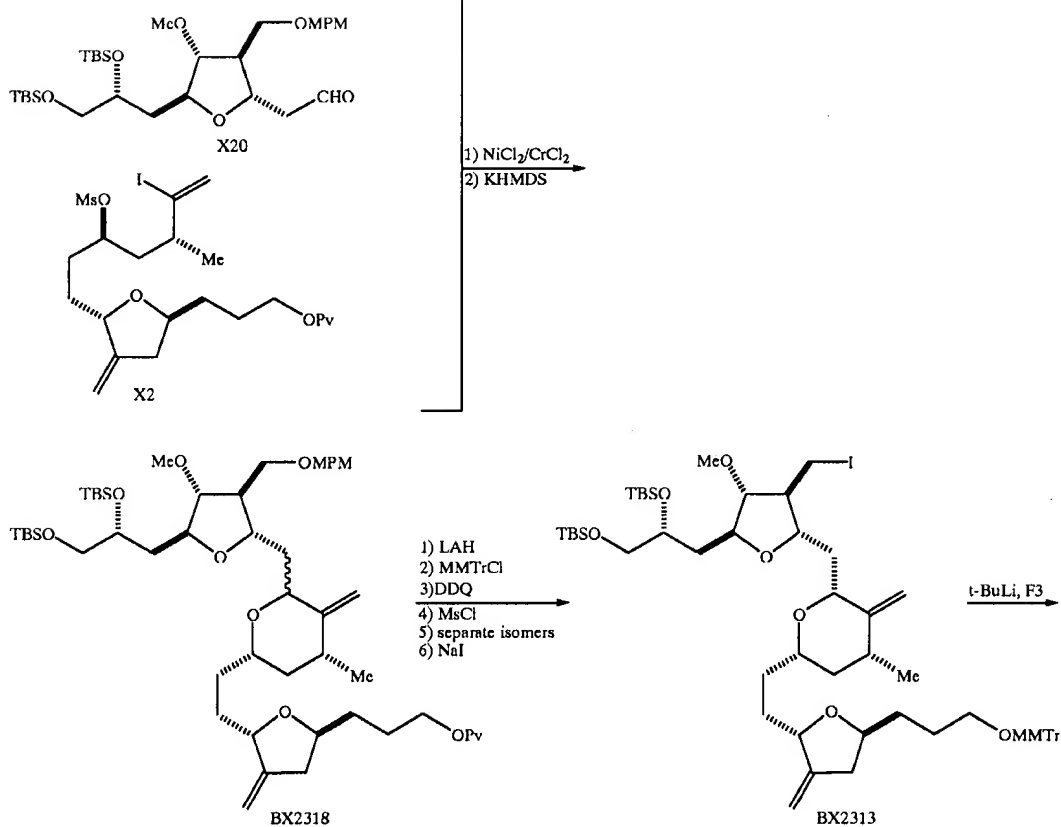
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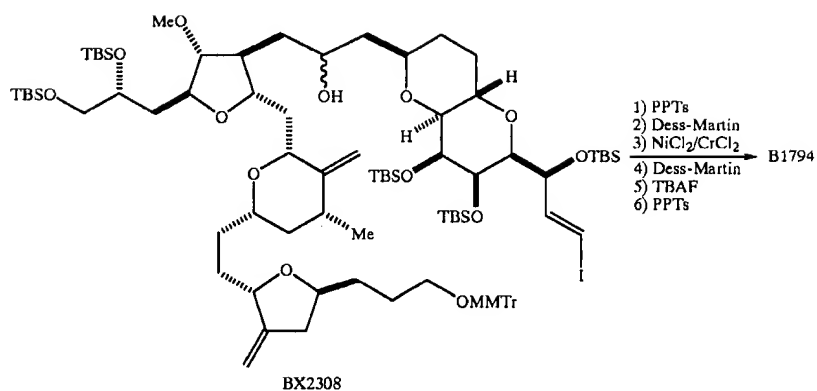
Scheme 6



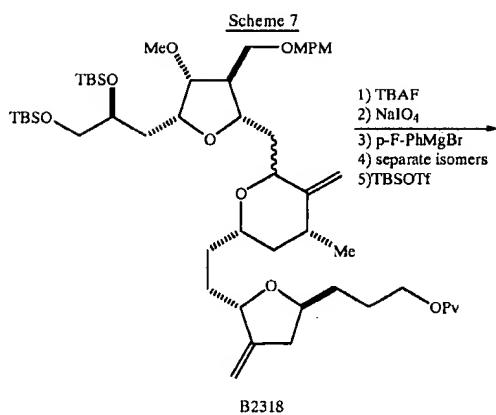
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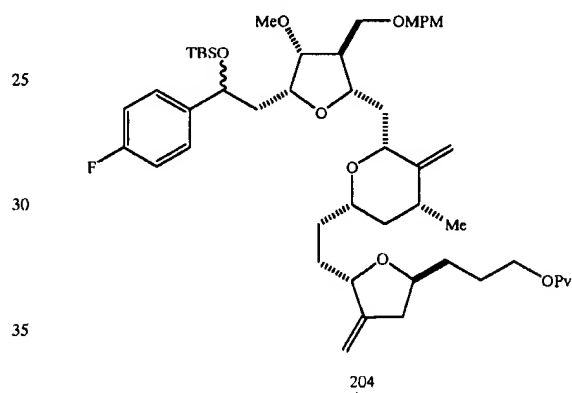
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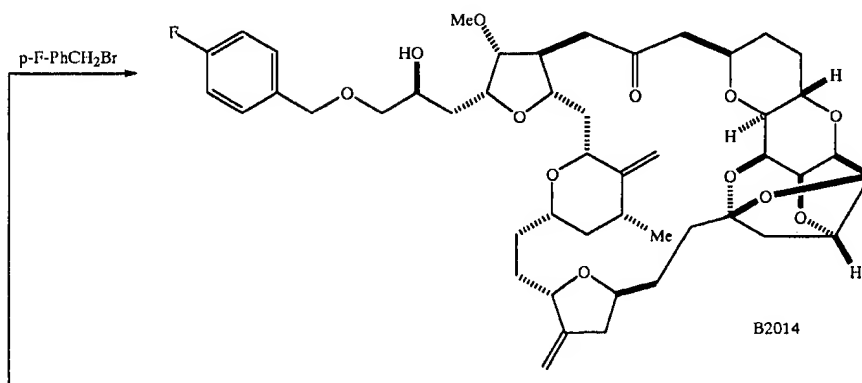
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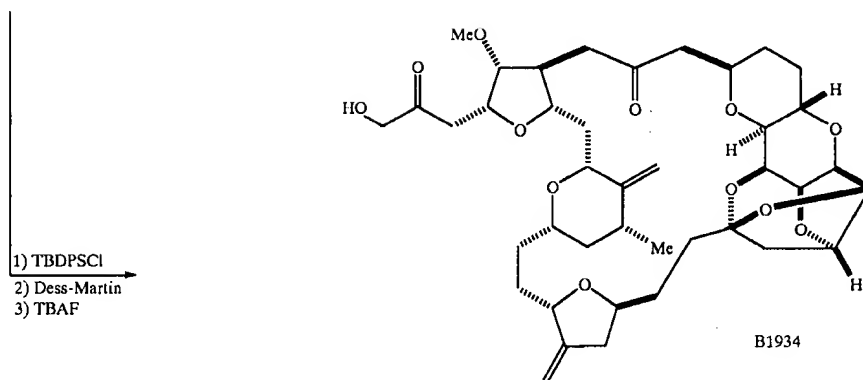
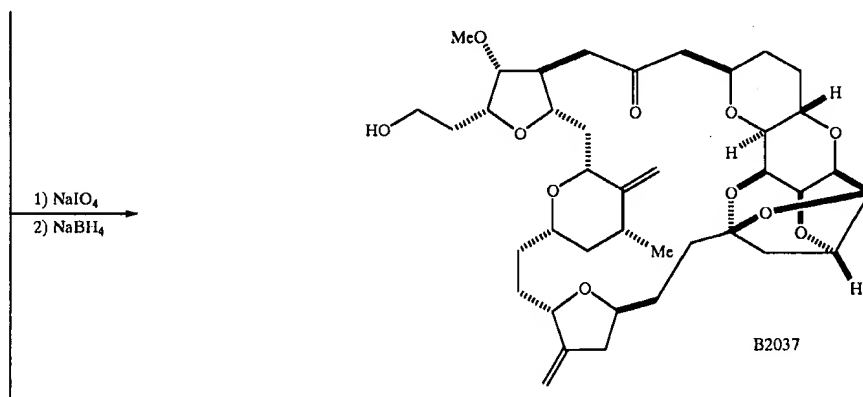
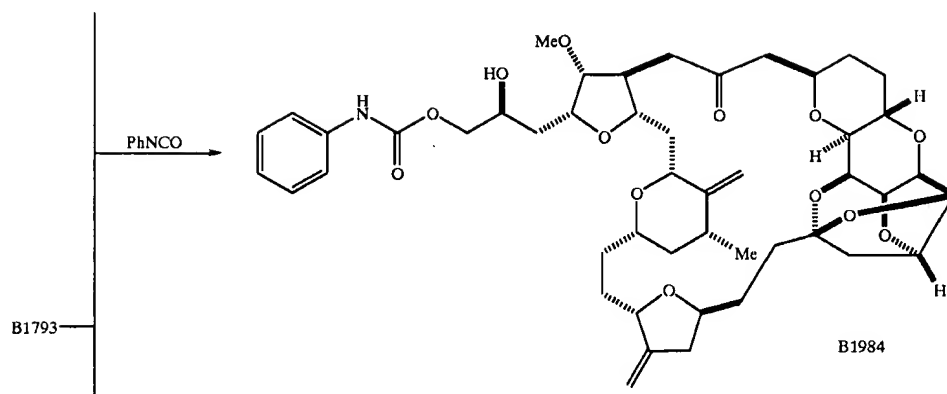
Scheme 8



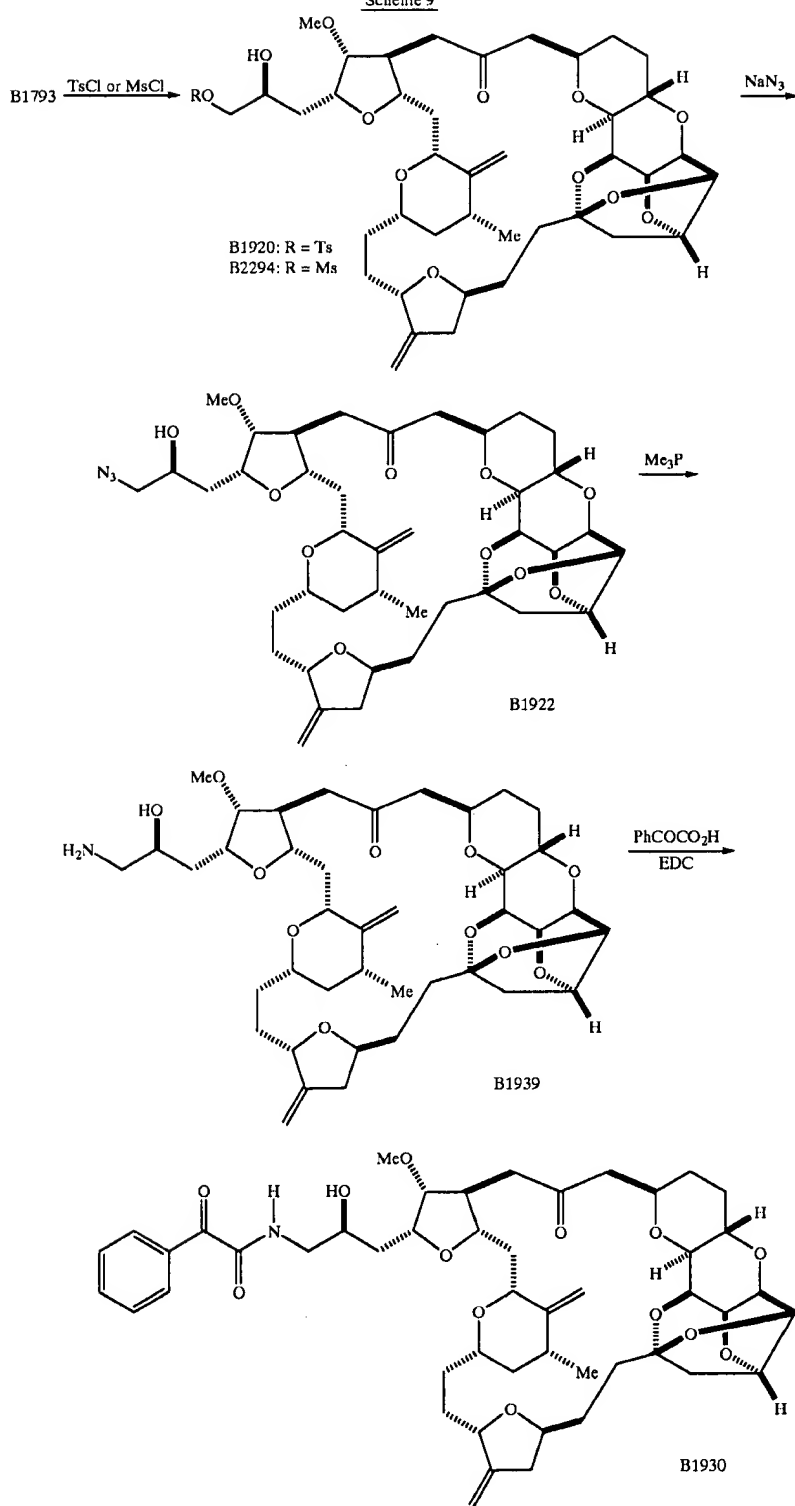
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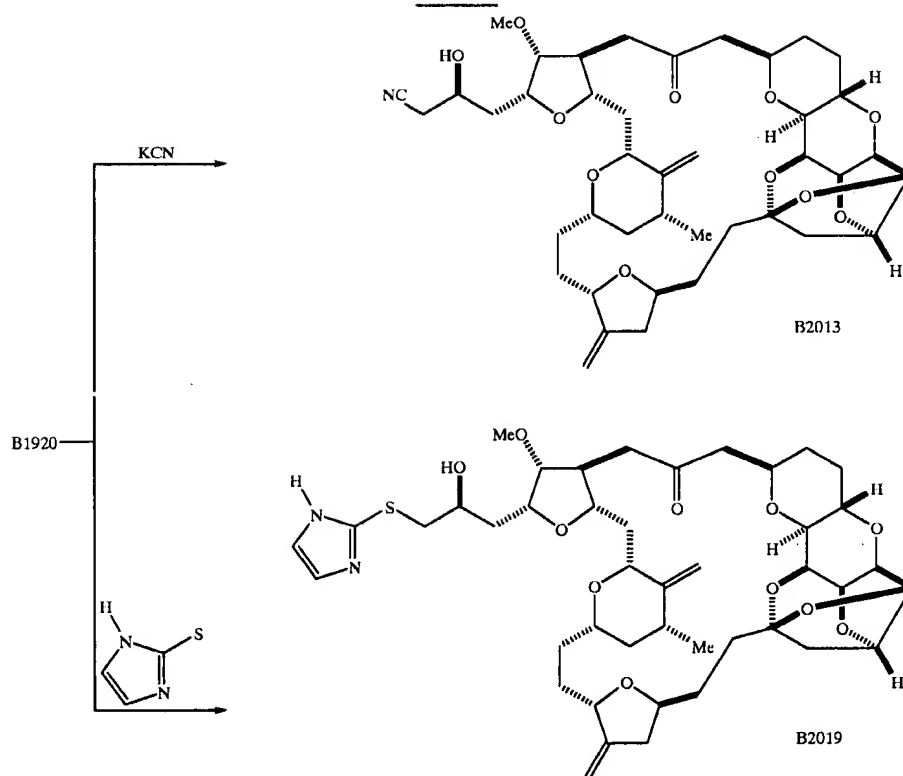
Scheme 9



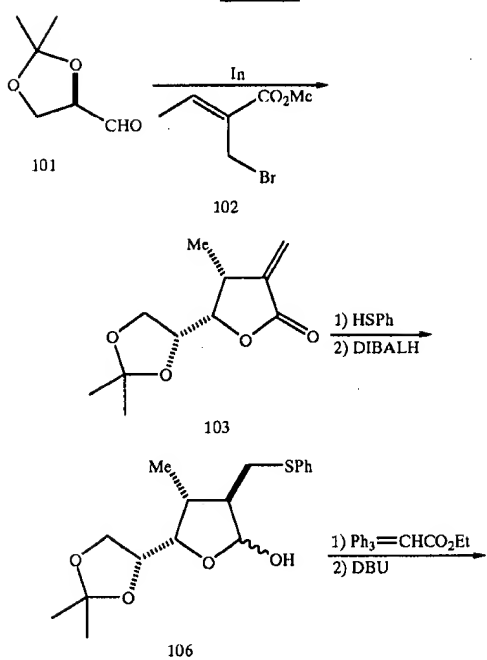
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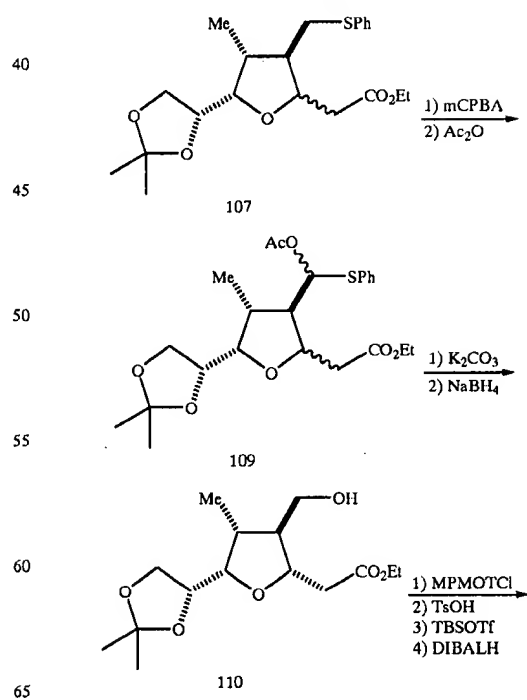
Scheme 10



Scheme 11

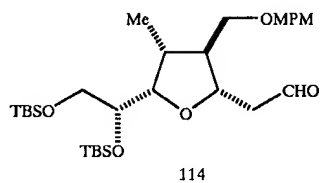


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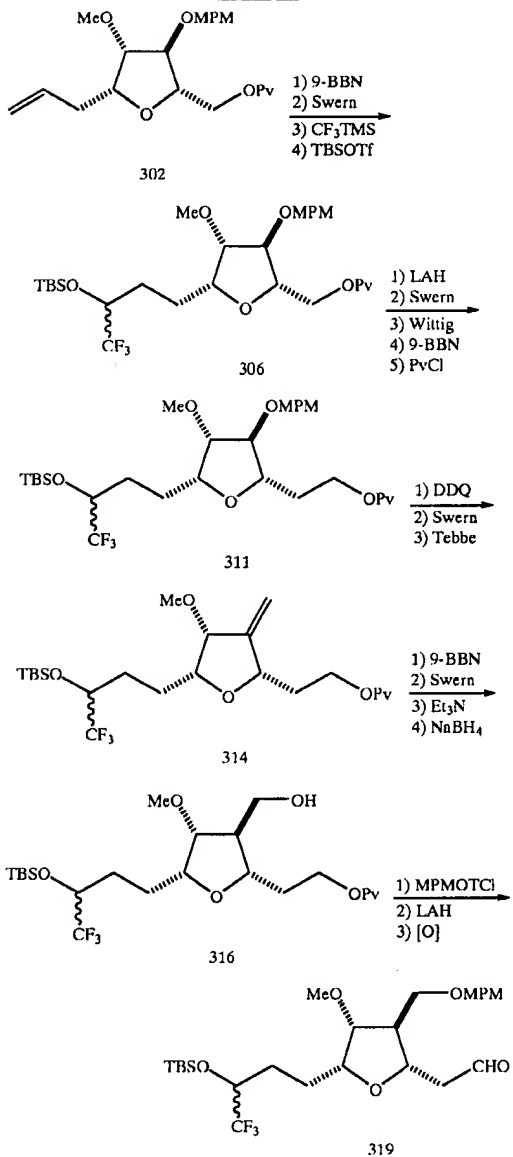


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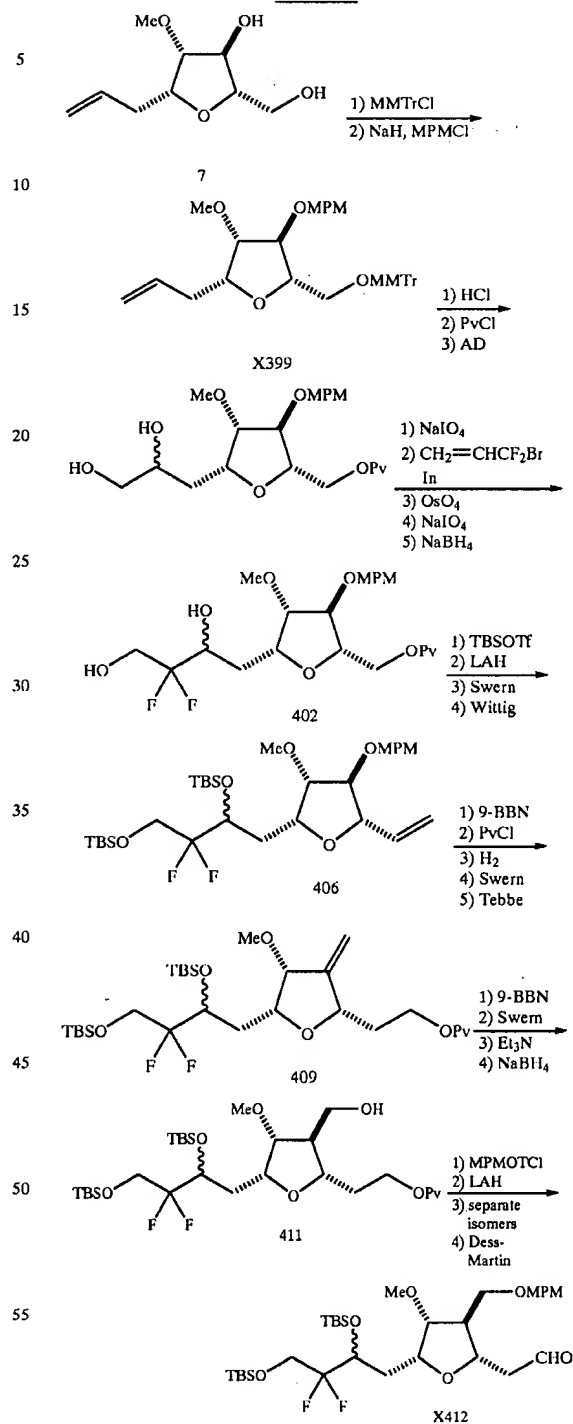


Scheme 12

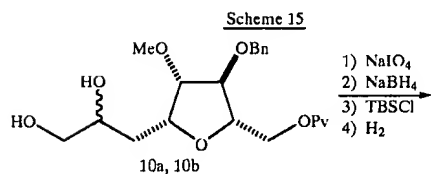
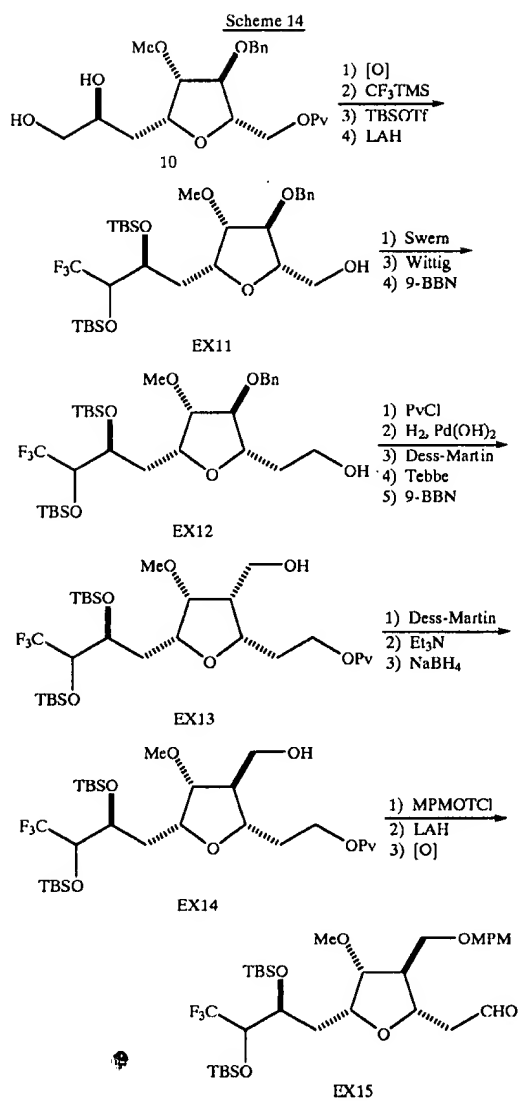


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Scheme 13

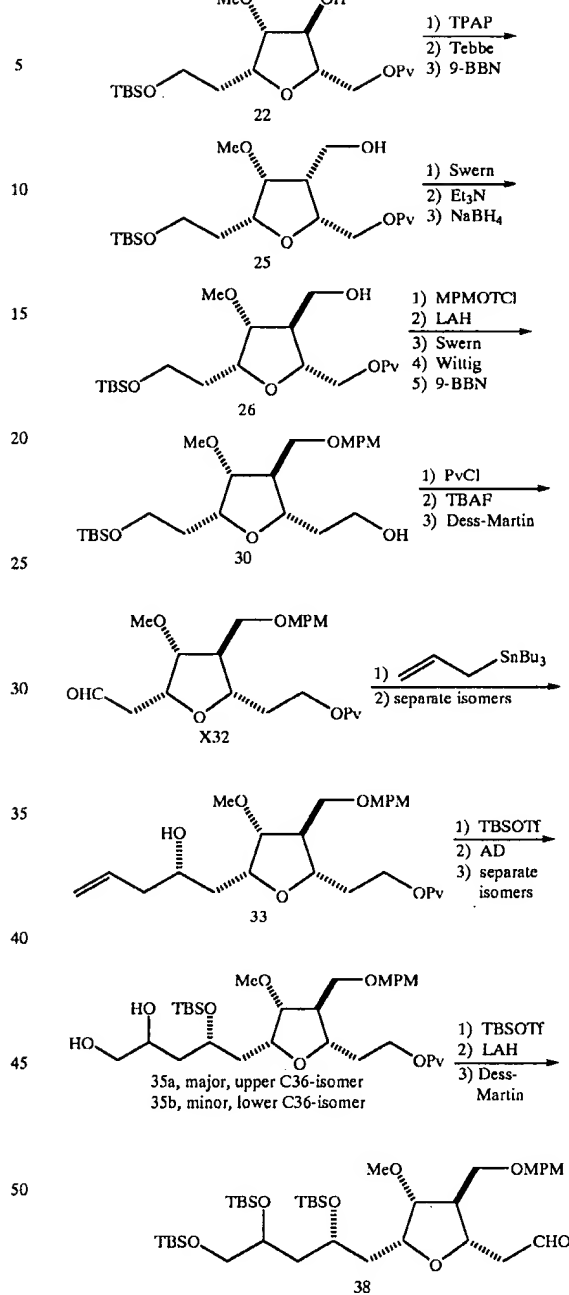


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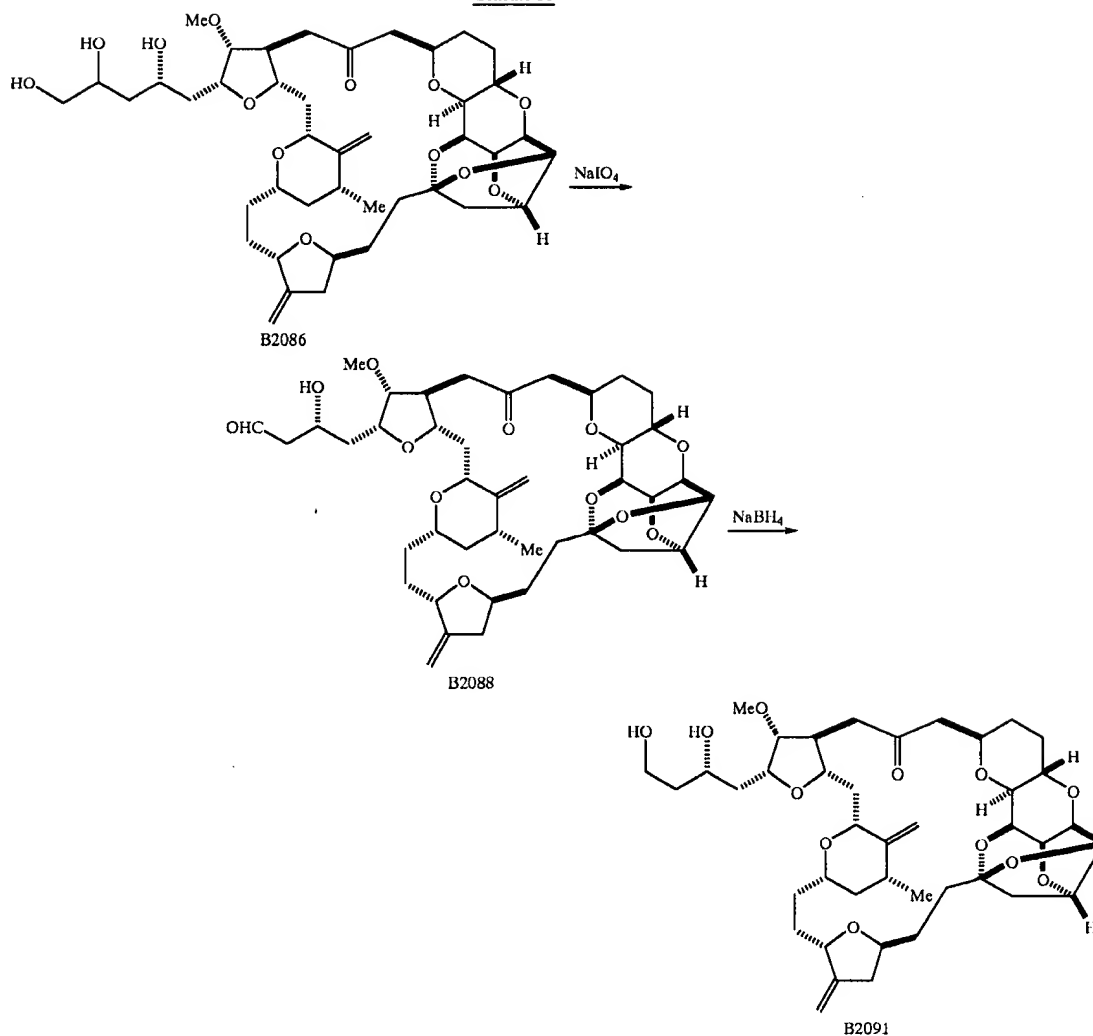
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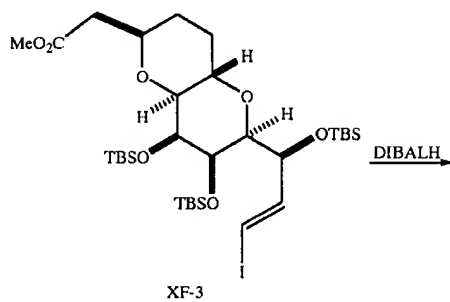
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Scheme 16

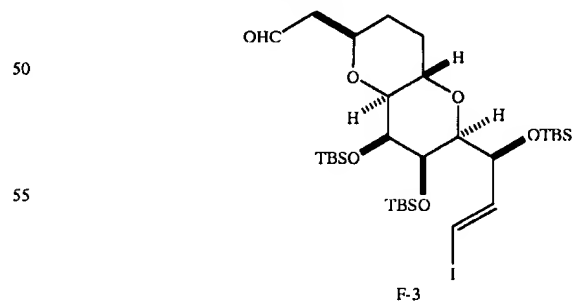


EXPERIMENTAL SECTION

Synthesis of Key Fragment F-3:



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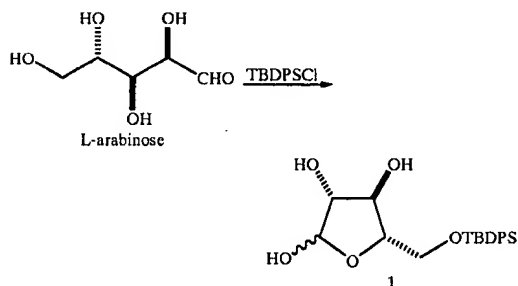


Key Fragment F-3. DIBALH (1 M in toluene, 3.86 mL) was added to a solution of XF-3 (1.46 g, 1.93 mmol) in toluene (37 mL) at -78°C . After stirring for 10 min, the reaction was quenched by careful addition of MeOH (0.46 mL) and H_2O (0.21 mL), warmed to rt and stirred for 15

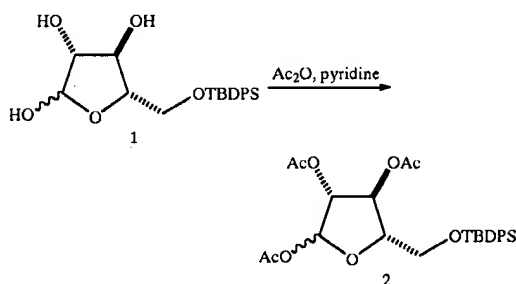
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min. The white suspension was filtered through Celite with 1:1 $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$. The filtrate was concentrated and purified by column chromatography (10% EtOAc-hexanes) to give key fragment F-3 (1.34 g, 96%) as an oil.

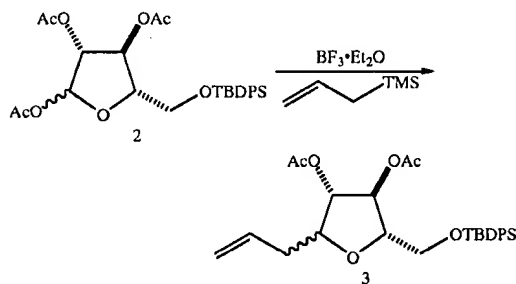
Synthesis of B1793:



Triol 1 A solution of TBDPSCl (444 mL, 1.7 mol) in DMF (0.5 L) was added in three portions to a suspension of L-arabinose (250.0 g, 1.66 mol), imidazole (231.4 g, 3.40 mol) and DMF (2.5 L). The addition of each portion took 1.5 h with a 30 min and a 15 h interval separating the second and third portions, respectively. The resulting solution was stirred for 3 h, concentrated and purified by flash chromatography (5% to 33% EtOAc-hexanes) to provide triol 1 (394 g, 61%).



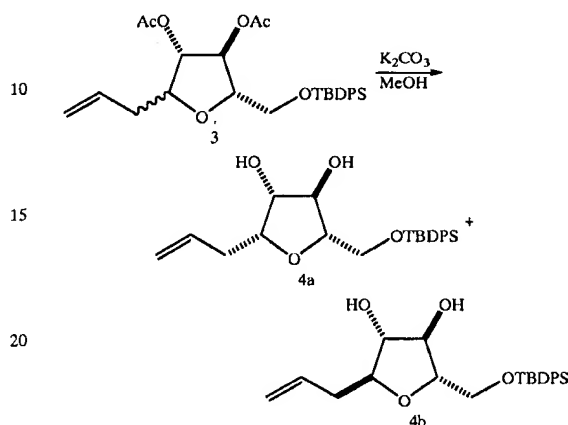
Triacetate 2 Acetic anhydride (6.06 mol) was added over 1.5 h to triol 1 (1.01 mol) in pyridine (1.0 L) at 15° C. The solution was stirred for 1 h, concentrated and purified by flash chromatography (15% to 25% EtOAc-hexanes) to afford triacetate 2 (518 g, 97%).



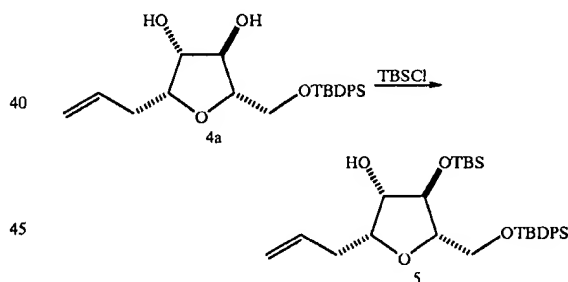
Diacetates 3 Allyltrimethylsilane (1.11 mol) followed by $\text{BF}_3 \cdot \text{OEt}_2$ (1.11 mmol) was added over 1.5 h to triacetate 2 (164 g, 0.32 mol) in toluene (1.5 L) at 0° C. The orange solution was stirred for 1 h at 0° C. and for 2 h at rt. The mixture was slowly poured into saturated aqueous NaHCO_3 (1.7 L) at 0° C. and stirred for 30 min. The separated

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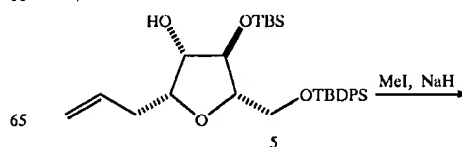
aqueous layer was extracted with EtOAc (3×600 mL) and the combined organic layers were dried over Na_2SO_4 , concentrated and purified by flash chromatography (5% to 10% EtOAc-hexanes) to furnish a mixture of diacetates 3 (108 g, 69%).



Diol 4a Solid K_2CO_3 (72 mmol) was added to diacetates 3 (108 g, 218 mmol) in MeOH (0.5 L) at rt. The suspension was stirred for 2.5 h and then concentrated. The orange residue was suspended in saturated aqueous NH_4Cl (150 mL), extracted with EtOAc (3×150 mL) and the combined organic layers were dried over Na_2SO_4 , concentrated and purified by flash chromatography (15% to 50% EtOAc-hexanes) to afford alpha-isomer 4a (33.86 g, 37%), and beta-isomer 4b (58 g, 63%).

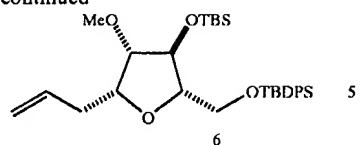


Alcohol 5 Imidazole (16.75 g, 246 mmol) and TBSCl (16.08 g, 107 mmol) were added to a solution of diol 4a (33.86 g, 82 mmol) in CH_2Cl_2 (250 mL) at 0° C. After 18 h at 0° C. and 5 h at rt, the reaction mixture was diluted with saturated aqueous NaHCO_3 (250 mL), stirred for 30 min and the layers were allowed to separate. The aqueous layer was extracted with EtOAc (3×250 mL) and the combined organic layers were dried over Na_2SO_4 , concentrated and purified by flash chromatography (2% to 50% EtOAc-hexanes) to furnish alcohol 5 (36.0 g, 83%).

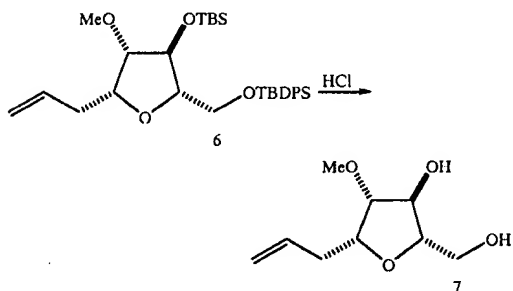


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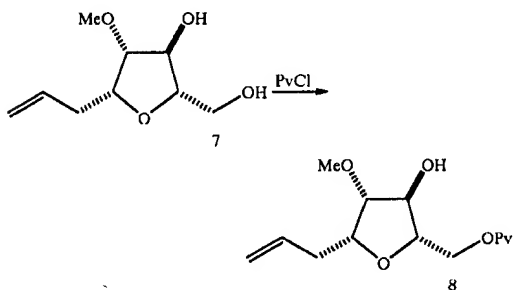
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Methyl ether 6 Iodomethane (16.5 mL, 265 mmol) and NaH (60% in mineral oil, 5.28 g, 132 mmol) were added to a solution of alcohol 5 (34.93 g, 66 mmol), THF (320 mL) and DMF (80 mL) at 0° C. After 19 h at 0° C., the reaction was quenched with saturated aqueous NH₄Cl and saturated aqueous Na₂S₂O₃. The resulting mixture was stirred for 20 min and the layers were allowed to separate. The aqueous phase was extracted with EtOAc (3x200 mL) and the combined organic layers were dried over Na₂SO₄, concentrated and purified by flash chromatography (3% EtOAc-hexanes) to afford methyl ether 6 (34.23 g, 96%).

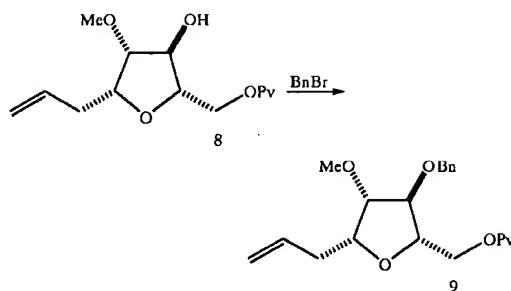


Diol 7 HCl (37% aqueous solution, 12.75 mL, 153 mmol) was added to a solution of methyl ether 6 (32.93 g, 61 mmol) in MeOH (110 mL) at rt. After 17 h, NaHCO₃ (17 g) was added to the reaction mixture. The mixture was stirred for 30 min, concentrated, suspended in EtOAc and filtered. The filtrate was concentrated and purified by flash chromatography (50% EtOAc-hexanes to EtOAc) to give diol 7 (10.0 g, 87%).

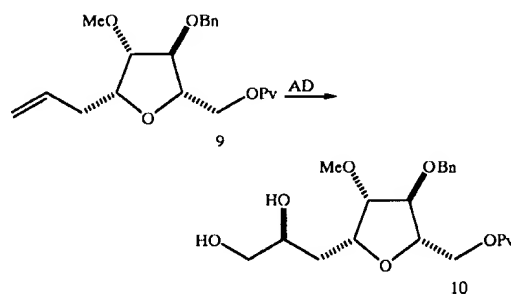


Alcohol 8 A solution of pivaloyl chloride (8.4 mL, 67 mmol) in pyridine (50 mL) was added over 1.5 h to a solution of diol 7 (12.24 g, 65 mmol) in pyridine (100 mL) at 0° C. After 1 h at 0° C. and 18 h at rt, the mixture was diluted with saturated aqueous NH₄Cl and extracted with EtOAc (3x800 mL). The combined organic layers were dried over Na₂SO₄, concentrated and purified by flash chromatography (50% EtOAc-hexanes) to furnish alcohol 8 (16.9 g, 96%).

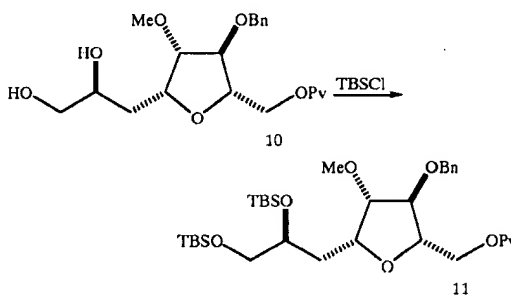
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Olefin 9 Benzyl bromide (62 mL, 521 mmol) and Bu₄NHSO₄ (10.6 g, 31 mmol) were added to a solution of alcohol 8 (16.9 g, 62 mmol) in CH₂Cl₂ (100 mL) at 0° C. A solution of NaOH (9.95 g, 248 mmol) in H₂O (10 mL) was added to the reaction mixture over 15 min. After 30 min at 0° C. and 18 h at rt, the reaction mixture was diluted with saturated aqueous NH₄Cl and extracted with CH₂Cl₂ (3x100 mL). The combined organic layers were dried over Na₂SO₄, concentrated and purified by flash chromatography (hexanes to 30% EtOAc-hexanes) to afford olefin 9 (22.1 g, 98%).



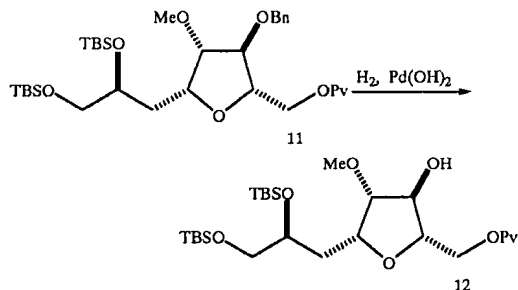
Diol 10 OsO₄ (0.1 M solution in toluene, 7.3 mL, 0.73 mmol) and a solution of olefin 9 (24.9 g, 69 mmol) in t-BuOH (165 mL) were added to a solution of K₂CO₃ (31.2 g, 161 mmol), K₃Fe(CN)₆ (74.4 g, 161 mmol), (DHQ)₂PYR (1.33 g, 1.50 mmol), H₂O (500 mL) and t-BuOH (330 mL) at 0° C. After 3 h at 0° C., Na₂S₂O₅·5 H₂O (37.3 g, 150 mmol) was added. The reaction mixture was warmed to rt, stirred for 1 h and extracted with EtOAc (3x300 mL). The combined organic layers were dried over Na₂SO₄, concentrated and purified by flash chromatography (5% isopropanol-CH₂Cl₂) to provide diol 10 (17.98 g, 75%).



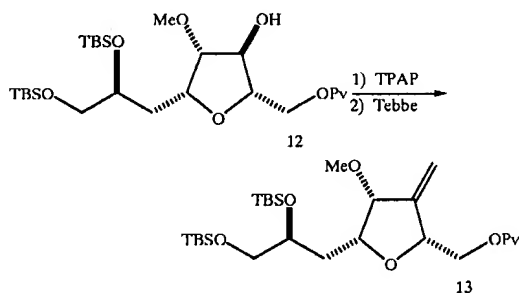
Silyl ether 11 Imidazole (21 g, 308 mmol) and TBSCl (26.5 g, 176 mmol) were added to a solution of diol 10 (17.4 g, 44 mmol) in DMF (90 mL) at rt. After 18 h, the reaction

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mixture was diluted with saturated aqueous NaHCO_3 (250 mL), stirred for 1 h and extracted with CH_2Cl_2 (3x100 mL). The combined organic layers were dried over Na_2SO_4 , concentrated and purified by flash chromatography (5% EtOAc-hexanes) to afford silyl ether 11 (25.7 g, 94%).



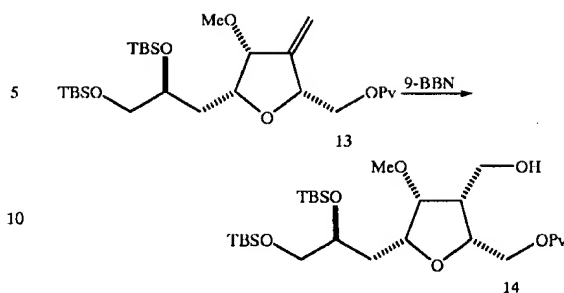
Alcohol 12 A mixture of silyl ether 11 (21.2 g, 33.8 mmol), $\text{Pd}(\text{OH})_2$ (20%, 4.7 g, 33.8 mmol) and EtOAc (200 mL) was stirred at rt under 1 atm H_2 for 3 h. The mixture was filtered through Celite, concentrated and purified by flash chromatography (10% to 20% EtOAc-hexanes) to afford alcohol 12 (17.4 g, 96%).



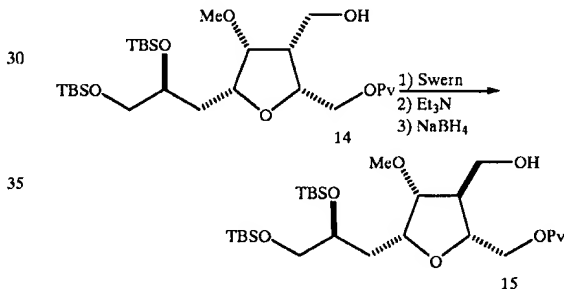
Olefin 13 4-Methylmorpholine N-oxide (7.66 g, 65 mmol) and TPAP (1.15 g, 3.26 mmol) was added in four portions over 20 min to a solution of alcohol 12 (17.4 g, 32.5 mmol) in CH_2Cl_2 (145 mL) at 0°C . After 20 min, the reaction mixture was diluted with Et_2O (50 mL) and saturated aqueous $\text{Na}_2\text{S}_2\text{O}_5$ (50 mL) and filtered through Celite. The organic layer was separated, washed sequentially with saturated aqueous CuSO_4 -brine (1:1) and brine, dried over Na_2SO_4 , filtered through Celite, and concentrated to afford the desired crude ketone.

Tebbe reagent was prepared by stirring bis (cyclopentadienyl)titanium (11.36 g, 45.6 mmol) and Me_3Al (2.0 M in toluene, 45.6 mL, 91.2 mmol) for 4 days at rt. This material was cooled to -25°C and a solution of crude ketone in THF (150 mL) was added. The reaction mixture was warmed to 0°C , stirred for 30 min, quenched by slow addition of 0.1 N NaOH (3.5 mL), and then stirred for an additional 20 min at rt. The mixture was diluted with Et_2O , filtered through Celite and concentrated. The residue was dissolved in CH_2Cl_2 , filtered through basic Al_2O_3 , concentrated and purified by flash chromatography (5% EtOAc-hexanes) to give olefin 13 (12.8 g, 74% for two steps).

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Alcohol 14 9-BBN (0.5 M in THF, 165 mL, 83 mmol) was added to a solution of olefin 13 (12.78 g, 24 mmol) in THF (280 mL) at 0°C . After stirring for 5 h at rt, the reaction mixture was recooled to 0°C at which time H_2O (200 mL), THF (100 mL) and $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$ (75 g) were added. The mixture was warmed to rt, stirred for 16 h and then concentrated. The aqueous residue was extracted with EtOAc (4x300 mL) and the combined organic layers were dried over Na_2SO_4 . Concentration and purification by flash chromatography (20% to 35% EtOAc-hexanes) afforded alcohol 14 (12.05 g, 91%).

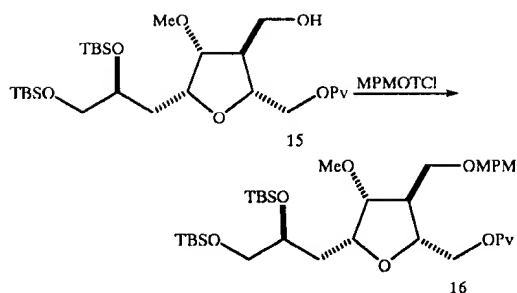


Alcohol 15 DMSO (9 mL, 127 mmol) was added to a solution of oxalyl chloride (5.6 mL, 64 mmol) in CH_2Cl_2 (350 mL) at -78°C . After stirring for 15 min, a solution of alcohol 14 (11.7 g, 0.021 mmol) in CH_2Cl_2 (50 mL) was added and stirring was continued for 1 h, after which Et_3N (26.7 mL, 192 mmol) was added. The reaction mixture was warmed to 0°C , stirred for 15 min, diluted with saturated aqueous NH_4Cl , and extracted with CH_2Cl_2 (3x200 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to furnish the desired crude aldehyde.

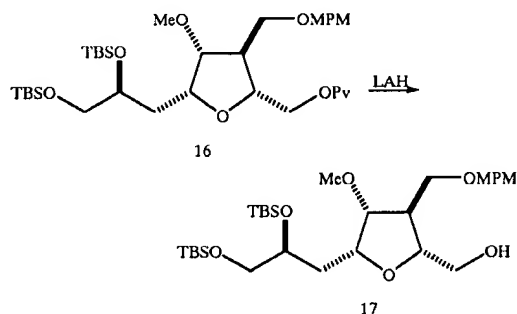
This material was dissolved in CH_2Cl_2 (200 mL) and treated with Et_3N (20 mL) at rt. After stirring overnight, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3x200 mL). The combined organic layers were dried over Na_2SO_4 , concentrated and filtered through a short SiO_2 column (20% EtOAc-hexanes) to afford the crude epimerized product.

The aldehyde was dissolved in Et_2O — EtOH (1:1, 100 mL), cooled to 0°C and treated with sodium borohydride (1.21 g, 32 mmol). The mixture was stirred for 20 min, carefully diluted with saturated aqueous NH_4Cl , stirred for 30 min at rt and extracted with CH_2Cl_2 (3x150 mL). The combined extracts were dried over Na_2SO_4 , concentrated and purified by flash chromatography (20% EtOAc-hexanes) to afford alcohol 15 (9.95 g, 85% for three steps).

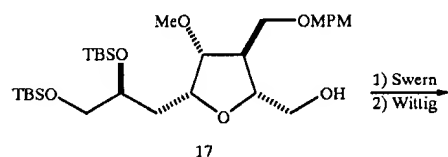
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MPM-ether 16 $\text{BF}_3 \cdot \text{OEt}_2$ (0.1 M in CH_2Cl_2 , 1.8 mL, 0.18 mmol) was added to a solution of alcohol 15 (9.87 g, 18 mmol), MPM-trichloroimidate (4.9 mL, 27 mmol) and CH_2Cl_2 (175 mL) at 0°C . After 40 min, a second portion of $\text{BF}_3 \cdot \text{OEt}_2$ (0.1 M in CH_2Cl_2 , 0.9 mL, 0.09 mmol) was added to the reaction mixture. After 20 min, the reaction was quenched with saturated aqueous NH_4Cl , stirred for 1 h at rt and diluted with Et_2O (600 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (150 mL). The combined organic extracts were washed sequentially with 0.1 N aqueous NaOH , saturated aqueous NaHCO_3 , brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (20% EtOAc-hexanes) to give MPM-ether 16 (10.20 g, 85%).

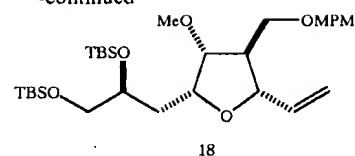


Alcohol 17 LAH (1 M in THF, 22.5 mL, 22.5 mmol) was added to a solution of MPM-ether 16 (10.05 g, 15 mmol) in Et_2O (1.0 L) at 0°C . After 30 min, the reaction was cautiously quenched with H_2O (1.3 mL), and 1 N aqueous NaOH (1.3 mL). After stirring for 1 h at rt, the suspension was filtered through Celite, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to afford alcohol 17 (8.18 g, 93%).



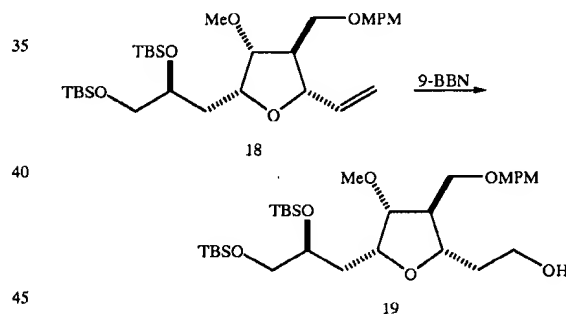
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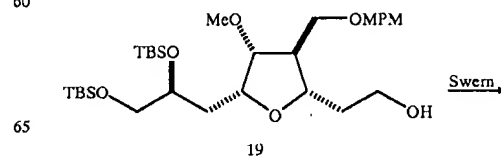


Olefin 18 DMSO (5.8 mL, 82.4 mmol) was added to a solution of oxalyl chloride (3.6 mL, 41.2 mmol) in CH_2Cl_2 (100 mL) at -78°C . After 15 min, a solution of alcohol 17 (7.94 g, 13.5 mmol) in CH_2Cl_2 (35 mL) was added to the reaction mixture. After stirring for 1 h, Et_3N (17 mL, 122 mmol) was added, the mixture was warmed to 0°C , stirred for 20 min, diluted with saturated aqueous NH_4Cl and then extracted with CH_2Cl_2 (3 \times 100 mL). The combined organic extracts were dried over Na_2SO_4 , concentrated and filtered through a short SiO_2 column (20% EtOAc-hexanes) to furnish the desired crude aldehyde.

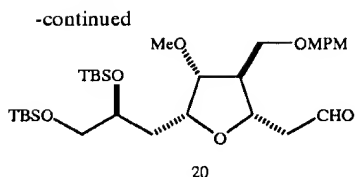
$n\text{-BuLi}$ (1.6 M, 20 mL, 30 mmol) was added dropwise to a solution of $\text{CH}_3\text{PPh}_3\text{Br}$ (10.1 g, 30 mmol) in THF (350 mL) and DMSO (100 mL) at 0°C . After 1 h, a solution of the crude aldehyde in THF (50 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (3 \times 500 mL). The combined extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (7% EtOAc-hexanes) to afford olefin 18 (5.57 g, 71% yield for 2 steps).



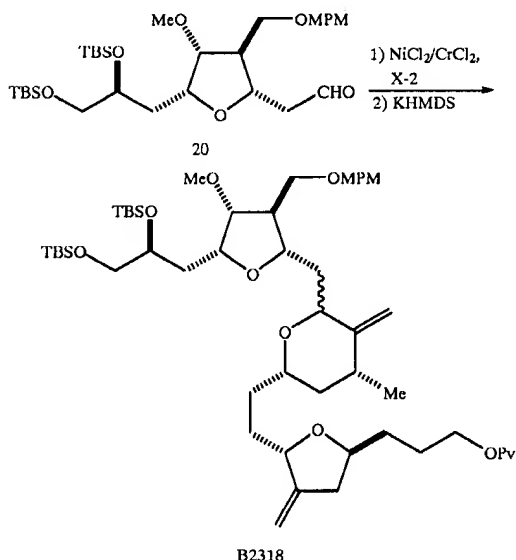
Alcohol 19 9-BBN (0.5 M in THF, 65 mL, 33 mmol) was added to a solution of olefin 18 (5.56 g, 9.6 mmol) in THF (85 mL) at 0°C . The mixture was stirred for 5 h at rt and then recooled to 0°C . H_2O (200 mL), THF (100 mL), and $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ (30 g) were sequentially added. After stirring overnight at rt, the organic volatiles were removed under reduced pressure. The aqueous residue was extracted with EtOAc (3 \times 200 mL) and the combined organic layers were dried over Na_2SO_4 . Concentration and purification by flash chromatography (30% EtOAc-hexanes) afforded alcohol 19 (12.05 g, 92%).



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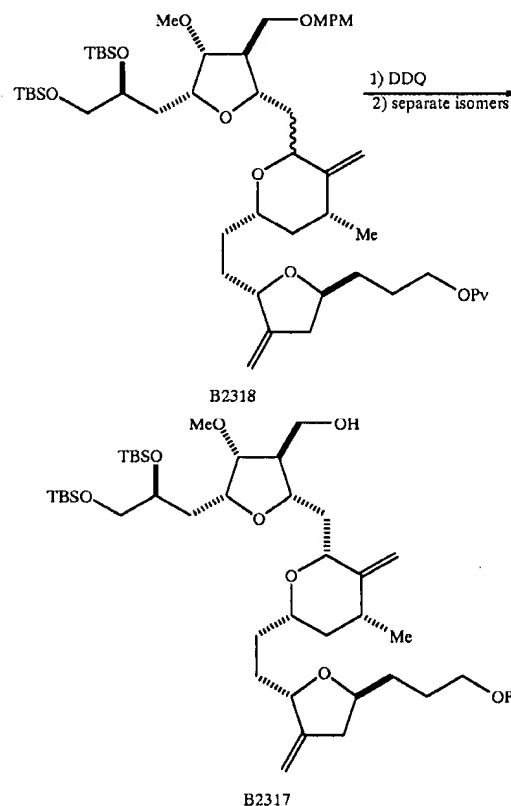
Aldehyde 20 DMSO (1.36 mL, 19.2 mmol) was added dropwise over 4 min to a solution of oxalyl chloride (1.26 mL, 14.4 mmol) in CH_2Cl_2 (120 mL) at -78°C . After stirring for 10 min, a solution of alcohol 19 (5.76 g, 9.61 mmol) in CH_2Cl_2 (20 mL) was added via cannula. The transfer was completed by rinsing with additional CH_2Cl_2 (2x5 mL). After stirring for 20 min, the mixture was treated with Et_3N (5.36 mL, 38.4 mmol) and stirred for 10 min at -78°C , 30 min at 0°C , and 10 min at rt. The reaction mixture was poured into saturated aqueous NaHCO_3 (200 mL) and the separated aqueous layer was extracted with CH_2Cl_2 (3x) followed by EtOAc (100 mL). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to furnish aldehyde compound 20 (5.28 g, 92%) as an oil.



B2318 0.1% $\text{NiCl}_2/\text{CrCl}_2$ (w/w, 3.21 g) and 1% $\text{NiCl}_2/\text{CrCl}_2$ (w/w, 4.31 g) was added to a solution of aldehyde 20 (3.73 g, 6.25 mmol), key fragment F-2 exemplified by vinyl iodide X2. (5.10 g, 9.16 mmol), THF (85 mL) and DMF (21 mL) at rt in a glove box. The reaction mixture was stirred for 24 h, removed from the glove box, cooled to 0°C , diluted with EtOAc (100 mL), quenched with saturated NH_4Cl (200 mL) and stirred for 30 min. The separated aqueous phase was extracted with EtOAc (6x) and the combined organic layers were dried over Na_2SO_4 concentrated and purified by column chromatography (20% to 30%) to give B2318 (~3 g) contaminated with close running impurities and the uncyclized intermediate (4.61 g). The latter (4.61 g, 4.48 mmol) was dissolved in THF (150 mL), cooled to 0°C and treated

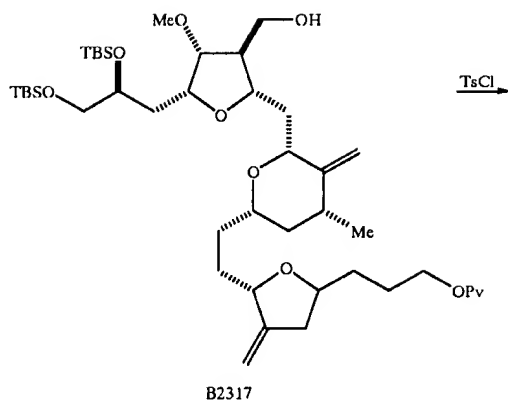
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with KHMDS (0.5 M in toluene, 14 mL, 7.0 mmol) over a 2 min period. After stirring at 0°C for 15 min, the reaction was quenched with saturated aqueous NH_4Cl (150 mL) and warmed to rt. The separated aqueous layer was extracted with EtOAc (3x) and the combined organic phases were dried over Na_2SO_4 , concentrated and combined with the partially purified product obtained above. Column chromatography (10% EtOAc-hexanes) afforded B2318 (3.17 g, 55%) as an inseparable ~3:1 mixture of C27 diastereomers.



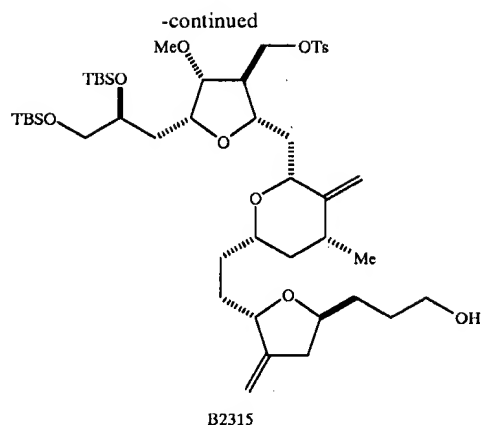
B2317 DDQ (1.45 g, 6.42 mmol) was added portion-wise over 30 min to a stirred solution of B2318 (3.12 g, 3.35 mmol) in CH_2Cl_2 (50 mL) and pH 7 phosphate buffer (5 mL) at rt. The reaction was quenched with saturated aqueous NaHCO_3 (50 mL), stirred for 5 min, diluted with additional saturated aqueous NaHCO_3 (100 mL), H_2O (200 mL) and extracted with Et_2O (5x). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (15% to 30% EtOAc-hexanes) to give recovered B2318 (1.40 g) and a mixture of the C27 isomeric products. The recovered B2318 was resubmitted to the reaction conditions described above to afford additional product. Recovered starting material was again cycled through the deprotection conditions. All of the desired material was combined and separated by MPLC to afford B2317 (1.65 g, 61%).

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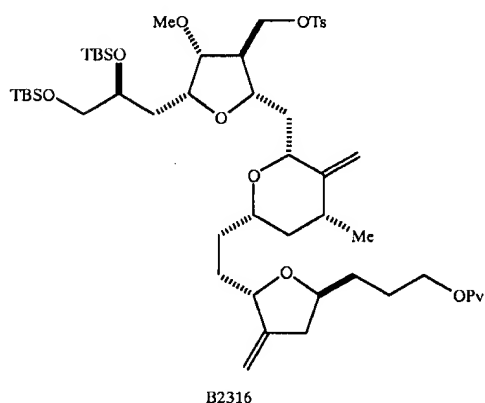
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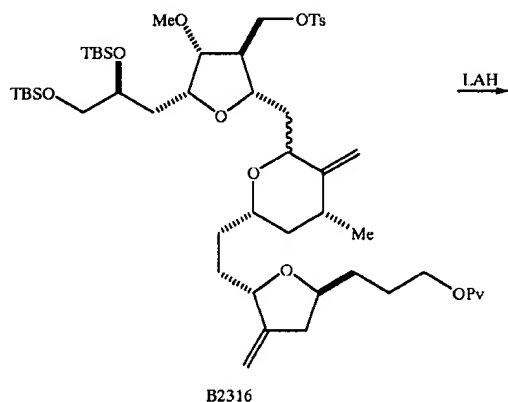


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B2316 TsCl (0.63 g, 3.30 mmol) was added to a solution of B2317 (1.60 g, 1.97 mmol) in CH_2Cl_2 (8 mL) and pyridine (2 mL) at rt. After stirring for 29 h, the reaction was quenched with saturated aqueous NaHCO_3 (30 mL) and H_2O (10 mL). The separated aqueous layer was extracted with Et_2O and the combined organic layers were dried over Na_2SO_4 , concentrated and purified by column chromatography (15% to 30% EtOAc-hexanes) to give B2316 (2.01 g, 92%) as an oil along with recovered B2317 (92 mg, 5.8%).



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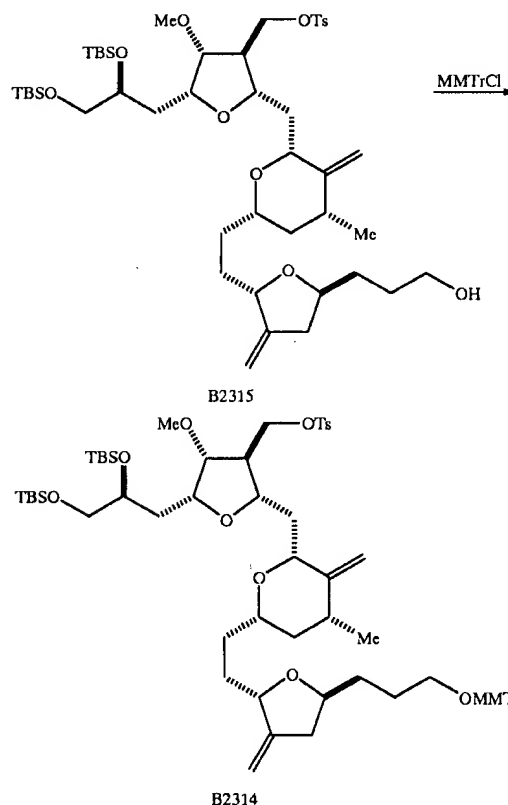
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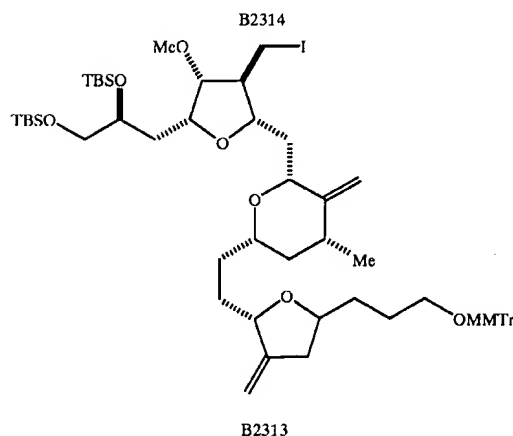
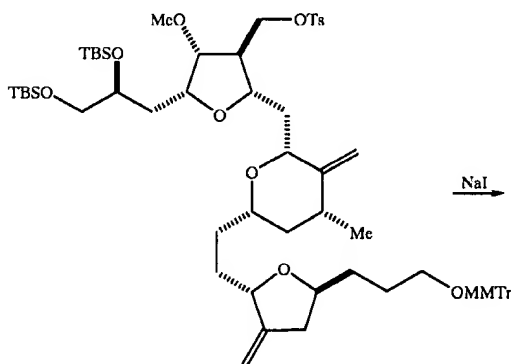


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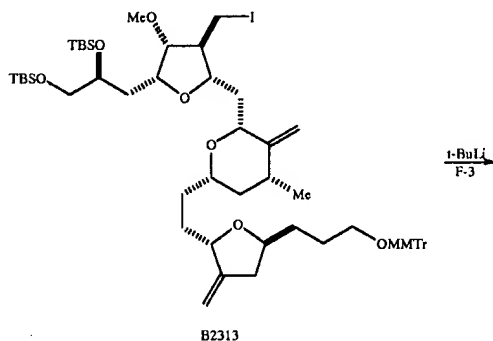
B2314 MMTTrCl (0.70 g, 2.26 mmol) was added to a solution of B2315 (1.33 g, 1.51 mmol) in CH_2Cl_2 (25 mL) and $i\text{Pr}_2\text{NEt}$ (0.79 mL, 4.53 mmol) at rt. The resulting mixture was stirred for 1 h and then poured into a mixture

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of saturated aqueous NaHCO_3 (20 mL), H_2O (10 mL) and Et_2O (50 mL). The separated aqueous layer was extracted with Et_2O (3 \times). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (CH_2Cl_2 followed by 15% to 30% EtOAc -hexanes) to give B2314 (1.65 g, 95%) as a solid foam.

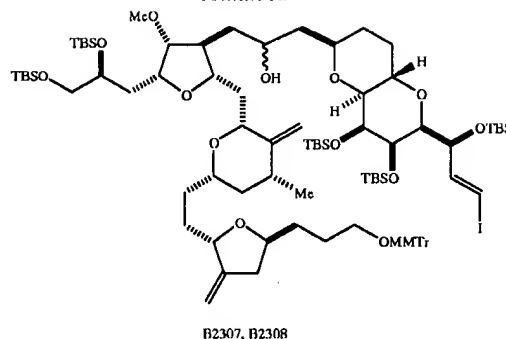


B2313 A mixture of B2314 (1.60 g, 1.39 mmol) and NaI (3.10 g, 20.8 mmol) in acetone (50 mL) was heated under reflux for 13 h. After cooling to rt, the reaction mixture was diluted with EtOAc , and concentrated. H_2O (5 mL), brine (20 mL) and $\text{Na}_2\text{S}_2\text{O}_3$ (200 mg) were added and the resulting mixture was extracted with Et_2O (4 \times). The combined extracts were dried over Na_2SO_4 , concentrated and purified by column chromatography (10% EtOAc -hexanes) to give B2313 (1.50 g, 97%) as an oil.

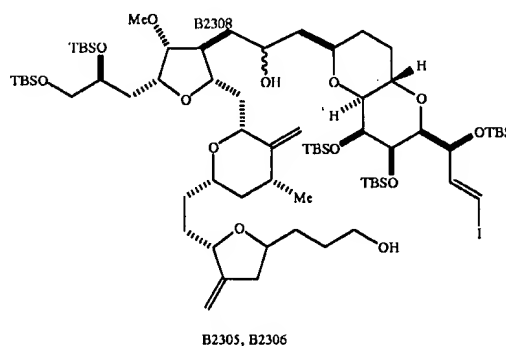
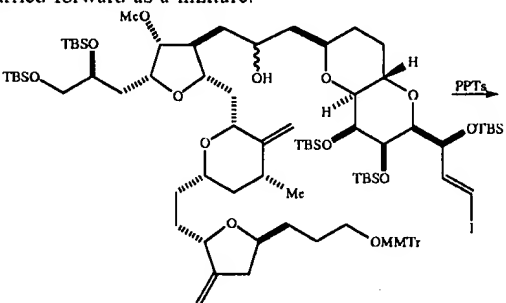


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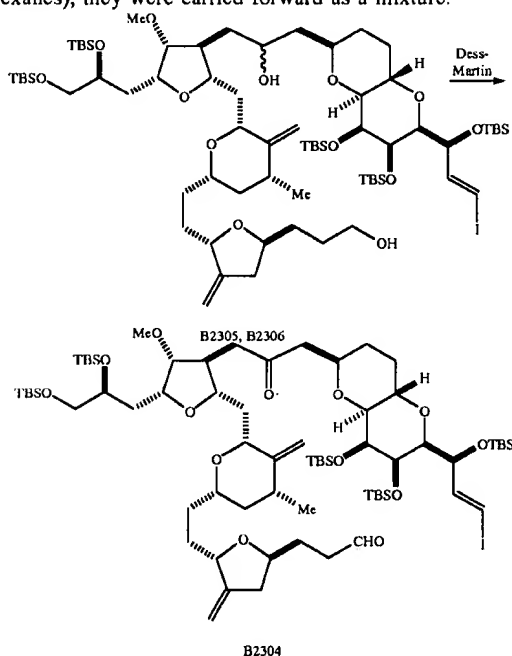
B2308 Tert-BuLi (1.7 M in pentane, 1.00 mL, 1.7 mmol) was added over 1 min to a solution of B2313 (0.90 g, 0.81 mmol) in Et_2O (14 mL) at -78°C . After stirring for 9 min, the mixture was transferred via cannula over 4 min to a solution of key fragment F-3 (0.83 g, 1.14 mmol) in Et_2O (4 mL) at -78°C . The transfer was completed by rinsing with additional Et_2O (2 mL). The resultant mixture was stirred at -78°C for 5 min and then at 0°C for 10 min, quenched with saturated aqueous NaHCO_3 (30 mL) and warmed to rt. The separated aqueous layer was extracted with Et_2O (3 \times) and the combined organic phases were dried over Na_2SO_4 and concentrated. The residue was combined with those of other two batches (corresponding to 0.11 g and 0.44 g of B2313) and purified by column chromatography (10% to 20% EtOAc -hexanes) to give a mixture of B2307 and B2308 (1.86 g, 83%) as a solid foam. Although the isomers could be separated by prep TLC (20% EtOAc -hexanes), they were carried forward as a mixture.



B2305 and B2306 The mixture of B2307/B2308 (1.80 g, 1.05 mmol) was dissolved in EtOH (20 mL), treated with PPTS (10.0 mg, 0.04 mmol), stirred at rt for 11 h and then quenched with NaHCO_3 (20.0 mg, 0.24 mmol). After stirring for 15 min, the mixture was concentrated, azeotroped with toluene (15 mL), and purified by column chromatography (20% to 30% EtOAc -hexanes) to give a mixture of B2305 and B2306 (1.22 g, 81%) as a solid foam. Although

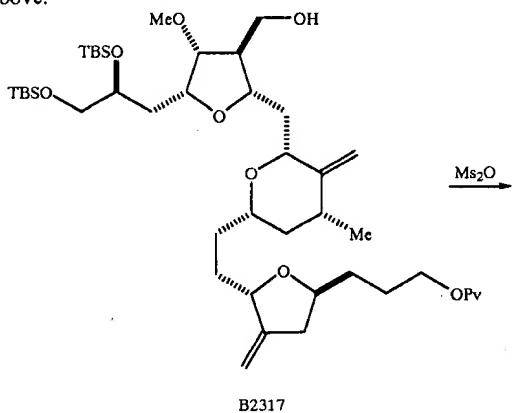
43

the isomers could be separated by prep TLC (30% EtOAc-hexanes), they were carried forward as a mixture.



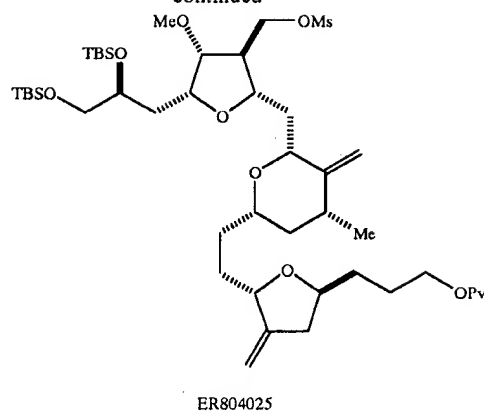
B2304 A mixture of B2305/B2306 (1.16 g, 0.68 mmol), and Dess-Martin periodinane (0.61 g, 1.44 mmol) in CH_2Cl_2 (35 mL) was stirred at rt for 1 h. Additional Dess-Martin periodinane (0.54 g, 1.27 mmol) was added to the mixture and stirring was continued for an additional 1 h. The mixture was diluted with Et_2O (100 mL), stirred for 20 min and filtered through Celite with Et_2O . The colorless filtrate was washed with saturated aqueous NaHCO_3 (100 mL) and the separated aqueous layer was extracted with Et_2O (3x). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (10% to 15% EtOAc-hexanes) to give B2304 (0.98 g, 84%) as a solid foam.

Alternatively, B2304 may be prepared as follows and in fact the synthesis described below is superior to that given above.

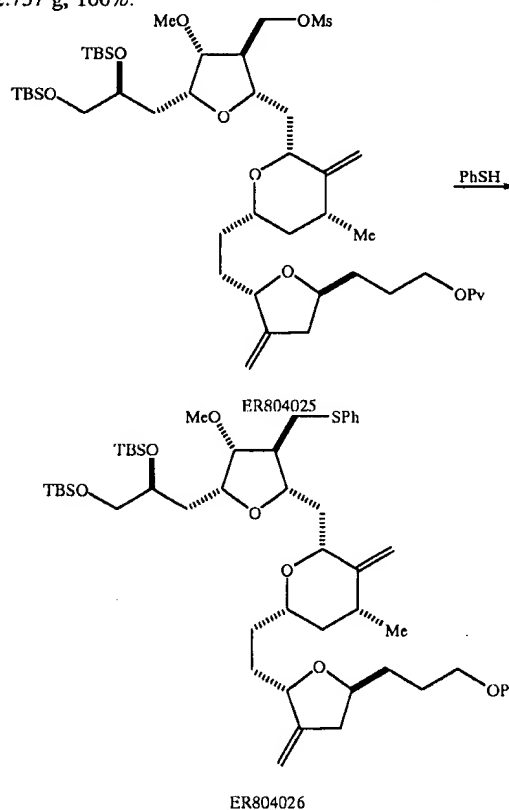


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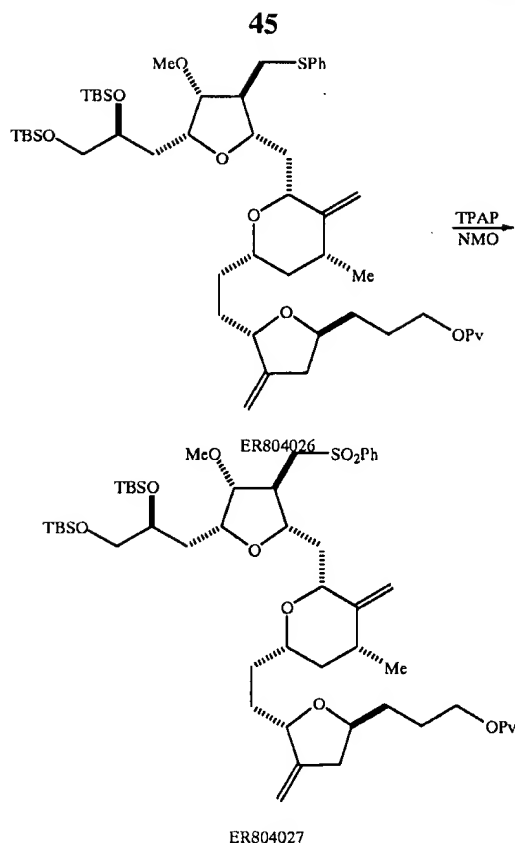
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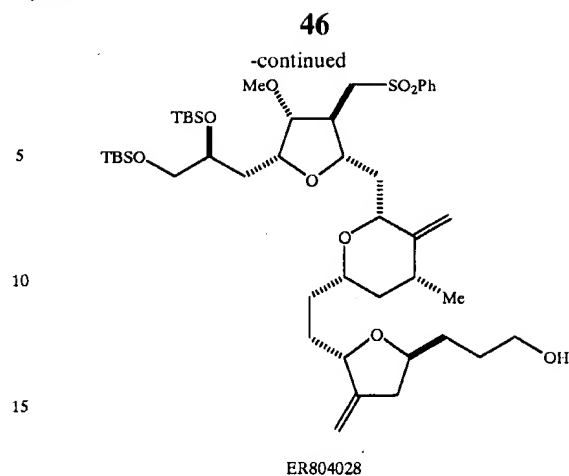
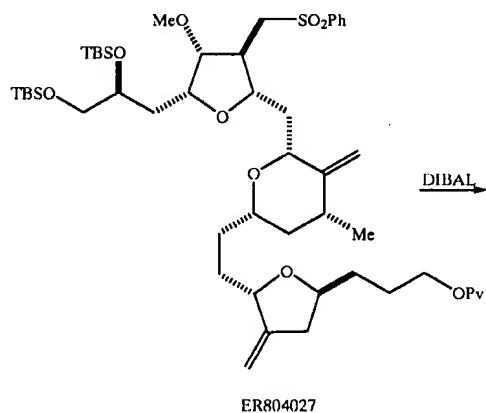
To a solution of the alcohol, 2.4 g mg, in methylene chloride, 29 mL, was added triflic anhydride, 770 mg. The mixture was stirred for 15 minutes, extracted with saturated sodium bicarbonate, dried and chromatographed to give 2.737 g, 100%.



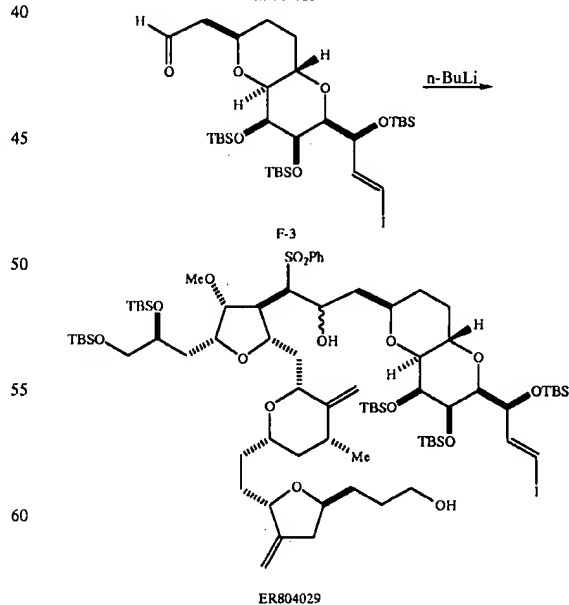
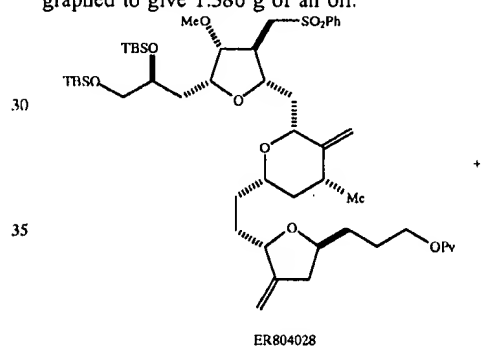
To a solution of the mesylate, 405 mg, in DMF, 0.06 mL, was added di-isopropylethylamine, 0.130 mL, followed by benzenethiol, 0.061 mL. After 4 hours and after 22 hours, additional amine, 0.03 mL, and benzenethiol, 0.015 mL, were added. After 24 hours, the mixture was diluted with 5% ethyl acetate/hexane, 1 mL and chromatographed to give 409 mg.



To a solution of the sulfide, 1.97 g, in acetonitrile, 16 mL, was added N-methylmorpholine oxide (NMO), and then a solution of 1.02 g, tetrapropylammonium perruthenate (VII), (TPAP), 38 mg, in acetonitrile, 1 mL. After 3.5 hours at room temperature, the mixture was heated to 40° C. for 1 hour. The mixture was cooled and aqueous satd. Sodium thiosulfate was added and the mixture partitioned between water and ethyl acetate. The usual work-up gave 1.567 g of a brown oil.

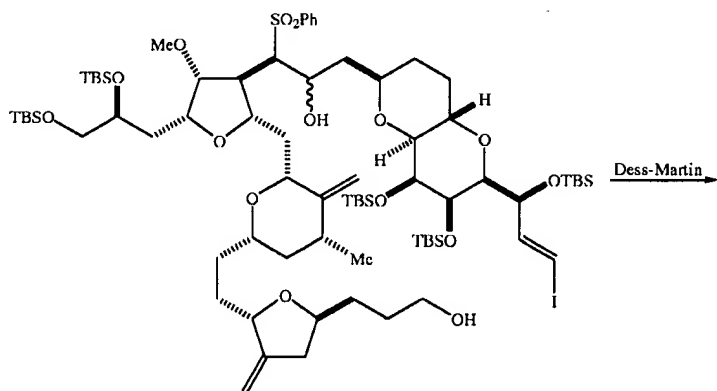


To a solution of the pivalate ester, 1.567 g, in methylene chloride, 11.2 mL, at -78° C. in was added DIBAL, 2.5 mL of a 1 M solution in toluene. After 15 minutes, additional DIBAL, 0.8 mL, was added. After an additional 5 minutes, methanol, 0.46 mL, was slowly added followed by water, 0.2 mL. The mixture was filtered through Celite and chromatographed to give 1.386 g of an oil.

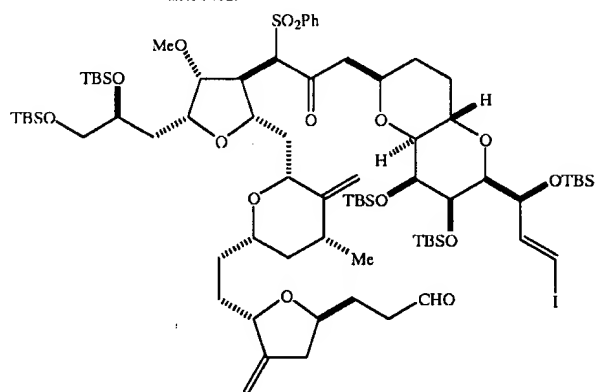


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To a solution of the sulfone, 36 mg, in DME, 1 mL, at -40°C . was added n-butyllithium, 2.8 equivalents. After 35 minutes, a solution of the aldehyde, 42 mg, in DME, 0.5 mL) was added. After 40 minutes, saturated aqueous ammonium chloride was added and the mixture extracted with ethyl acetate. The usual work-up, followed by chromatography gave 52 mg of an oil.



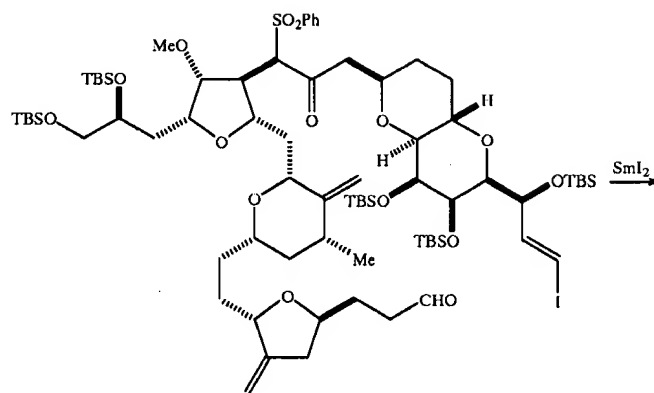
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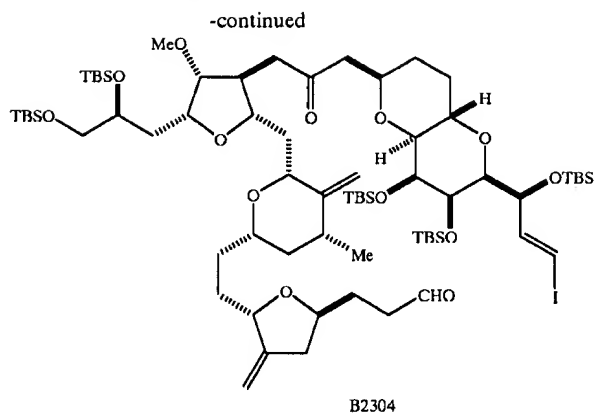
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To a solution of the alcohol, 42 mg, in methylene chloride, 2 mL, was added the Dess Martin reagent, 36.4 mg. The mixture was stirred for 30 minutes and ether was added. The mixture was filtered through Celite, washed with saturated sodium bicarbonate, with saturated sodium thiosulfate, worked up in the usual way and chromatographed to give 38 mg of an oil.



ER804030



Preparation of SmI_2 Solution

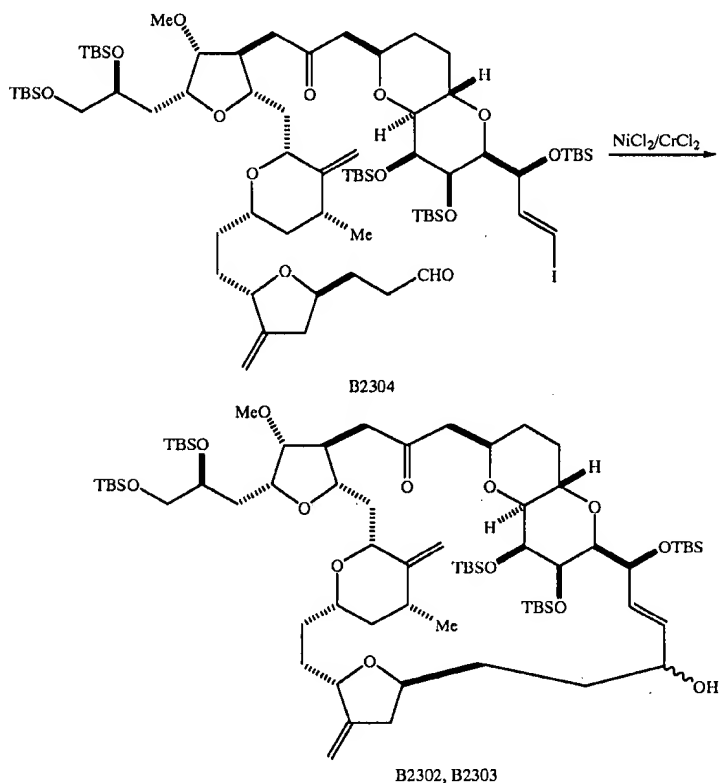
A solution of 1,2-di-iodoethane in 10 mL of THF was added to a suspension of Sm , 0.16 g, in THF, 1 mL. The mixture was stirred for 1 hour.

An aliquot of this solution, 0.03 mL, was added to a solution of the sulfone in THF at -78°C . After 5 minutes, additional SmI_2 reagent, 0.05 mL, was added. After a few additional minutes, more reagent, 0.25 mL, was added. The cooling bath was removed and saturated aqueous sodium bicarbonate, 3 mL, was added. The mixture was partitioned between ether and water and the usual work-up gave 9.1 mg, 81%, of an oil.

20 B2302 and B2303 In a glove box, $\text{NiCl}_2/\text{CrCl}_2$ (1% w/w, 1.09 g, 8.86 mmol) was added to a solution of B2304 (1.01 g, 0.70 mmol) in THF (600 mL) and DMF (150 mL) at rt. After stirring for 2 days the reaction mixture was taken out of the glove box, cooled to 0°C , quenched with saturated aqueous NH_4Cl (300 mL) and stirred at 0°C for 20 min.

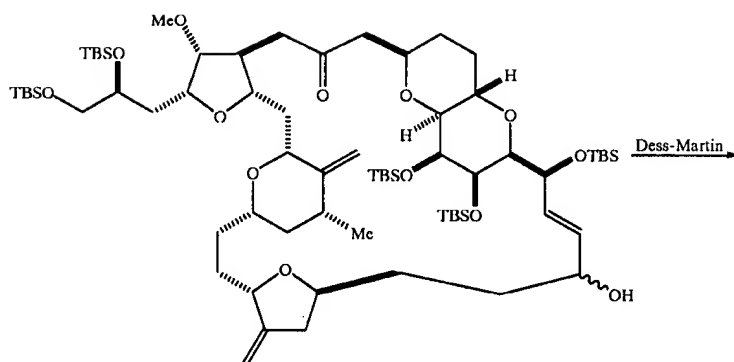
25 After addition of H_2O (100 mL), the two layers were separated and the aqueous layer was extracted with EtOAc (5x). The combined organic phases were washed with brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (15% EtOAc-hexanes) to furnish a mixture of B2302 and B2303 (0.84 g, 92%) as a solid foam.

30 Although the isomers could be separated by prep TLC (20% EtOAc-hexanes), they were carried forward as a mixture.

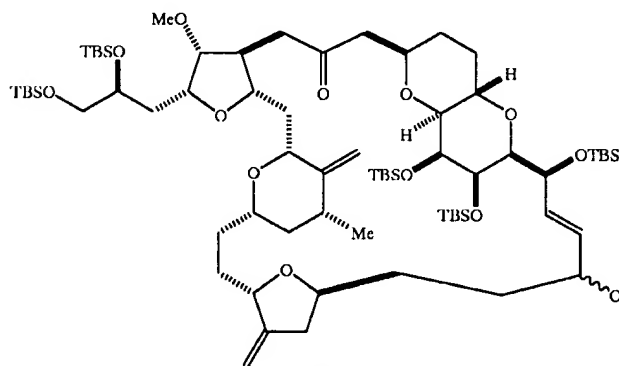


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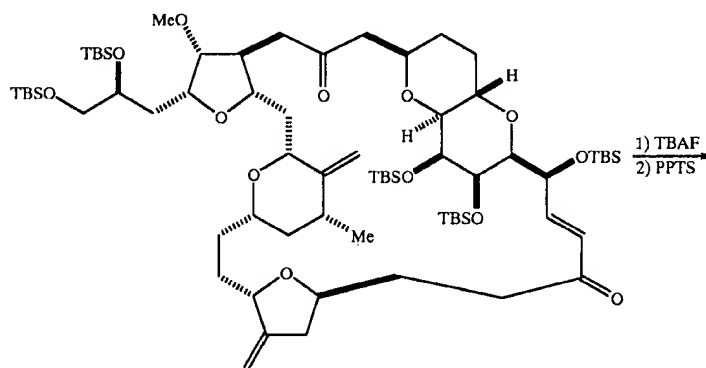
B2302, B2303



B2301

B2301 A mixture of B2302/B2303 (0.79 g, 0.60 mmol) and Dess-Martin periodinane (0.26 g, 0.60 mmol) in CH_2Cl_2 (30 mL) at rt was stirred for 30 min. Additional Dess-Martin periodinane (0.26 g, 0.60 mmol) was added to the mixture and stirring was continued for additional 1.5 h. The mixture was then diluted with Et_2O (100 mL), stirred for 15 min and

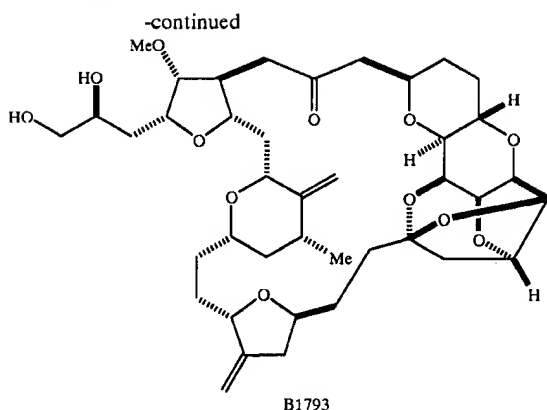
³⁵ filtered through Celite. The filtrate was washed with saturated aqueous NaHCO_3 (100 mL) and the separated aqueous layer was extracted with Et_2O (3 \times). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (10% to 15% EtOAc -hexanes) to give B2301 (0.67 g, 85%) as an oil.



B2301

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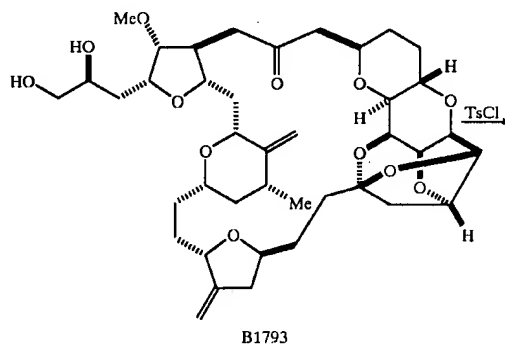
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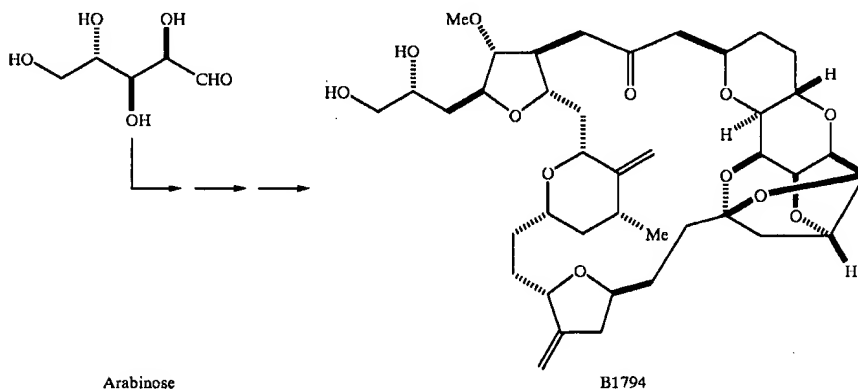
B1793 TBAF (1 M in THF containing 0.5 M imidazole HCl, 4.60 mL, 4.60 mmol) was added over 2 min to a solution of B2301 (0.62 g, 0.48 mmol,) in THF (29 mL) at rt and the resulting mixture was stirred for 18 h. After dilution with hexanes (10 mL), the reaction mixture was directly loaded onto a SiO₂ column packed with 50% EtOAc-hexanes and eluted with 50% EtOAc-hexanes (1 L) followed by 10% MeOH/EtOAc to collect a mixture of intermediates. After solvent removal, the residue was dissolved in CH₂Cl₂ (15 mL) and treated with PPTS (645 mg). After stirring for 1 h at rt, additional PPTS (414 mg) was added and the resulting white suspension was stirred for 4.5 h. The reaction mixture was then directly loaded onto a SiO₂ column packed with 70% EtOAc-hexanes and eluted with 70% EtOAc/hexanes (0.5 L), EtOAc (1 L). Elution with 5% to 10% MeOH/EtOAc furnished pure B1793 (181 mg) and elution with 15% MeOH—EtOAc gave additional semi-pure product, which after purification by preparative TLC (10% MeOH—EtOAc) provided additional pure B1793 (42 mg). B1793 (total 223 mg, 64%) was obtained as a white solid. HRMS: calcd for C₄₀H₅₈O₁₂+Na 753.3826. Found: 753.3808.

B1794 Except for stereochemical and protecting group differences (Schemes 3 and 5), arabinose was converted to B1794 in a manner similar to that described for B1793 (see schemes 4 and 5). HRMS: calcd for C₄₀H₅₈O₁₂+Na 753.3826. Found: 753.3856.

Synthesis of Representative B1793 Analogs:

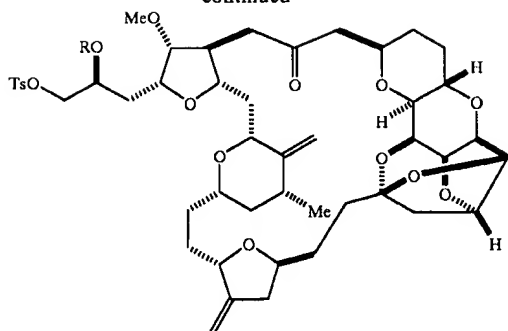


Synthesis of B1794:



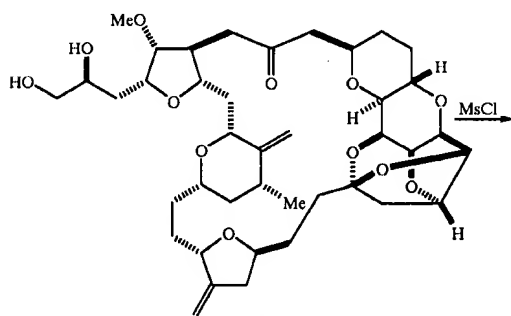
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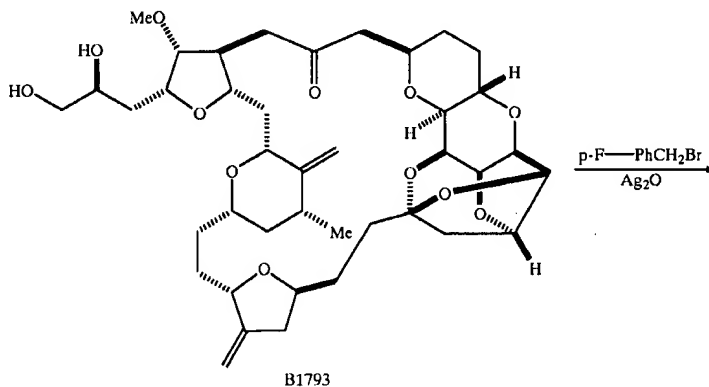
B1920: R = H
B1921: R = Ts

B1920 and B1921 TsCl (9.9 mg, 0.052 mmol) was added to a solution of diol B1793 (7.6 mg, 0.010 mmol) in CH_2Cl_2 (1 mL) and pyridine (0.1 mL) at rt. After 48 h, the reaction was quenched with a 1:4 mixture of saturated aqueous NaHCO_3 -brine and extracted with CH_2Cl_2 (4x). The combined extracts were dried over Na_2SO_4 and concentrated. Purification by preparative TLC (80% EtOAc-hexanes) afforded monotosylate B1920 (6.0 mg, 67%), and ditosylate B1921 (1.8 mg, 18%).



B1793

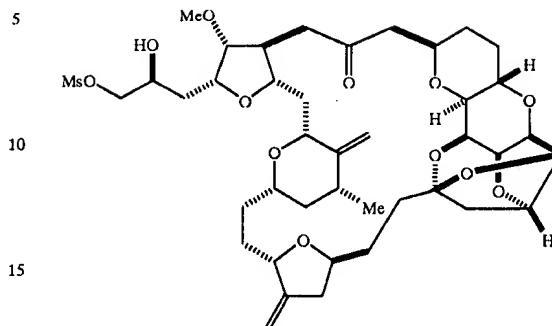
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B1793

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B2294

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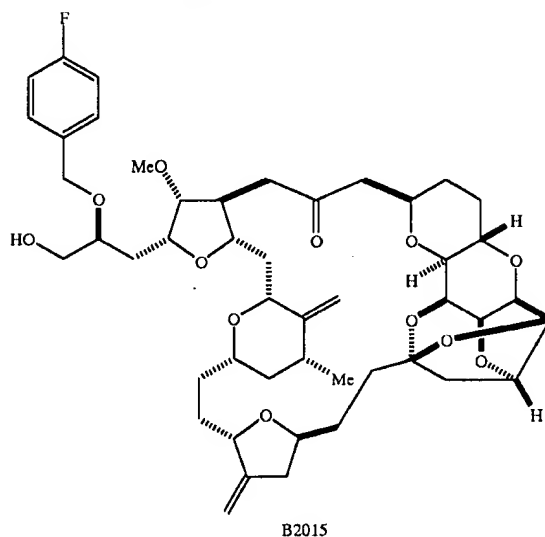
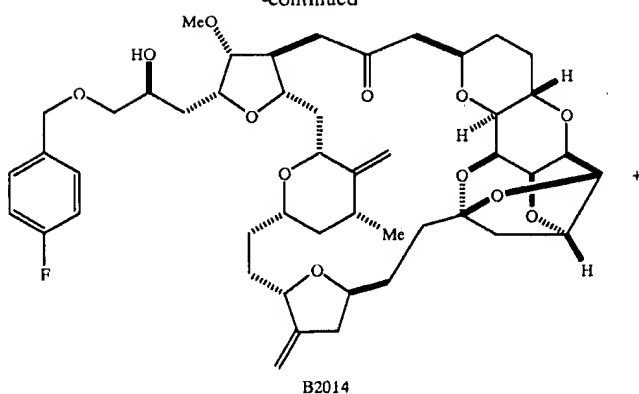
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B2294 MsCl (0.3 M in CH_2Cl_2 , 98 μL , 0.030 mmol) was added dropwise over 40 min to a mixture of collidine (7 μL , 0.054 mmol), B1793 (20.8 mg, 0.028 mmol) and CH_2Cl_2 (1 mL) at 0°C . After 76 h at 4°C , the reaction was quenched with a 1:4 mixture of saturated aqueous NaHCO_3 -brine and extracted with CH_2Cl_2 (4x). The combined extracts were dried over Na_2SO_4 and concentrated. The crude product was dissolved in toluene (3 mL) concentrated and purified by preparative TLC (1.5% MeOH-EtOAc) to afford mesylate B2294 (21.4 mg, 95%).

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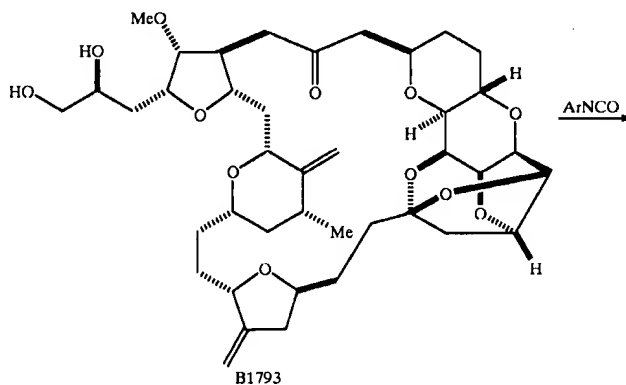
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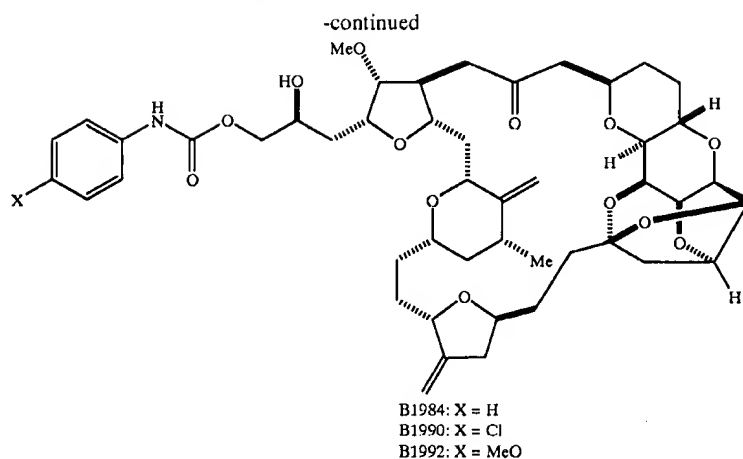
B2014 and B2015 A 0.016 M solution of 4-fluorobenzyl bromide in Et₂O (800 μ L, 13 μ mol) and Ag₂O (10 mg, 43 μ mol) were each added in three portions at 1 h intervals to a rt solution of B1793 (1.7 mg, 2.3 μ mol) in Et₂O (1.2 mL). The mixture was protected from light, stirred for 7 h and

then filtered through Celite. Concentration and purification by preparative TLC (EtOAc) afforded primary ether B2014 (1.1 mg, 56%), and secondary ether B2015 (0.6 mg, 31%). HRMS (FAB): calcd for C₄₇H₆₃FO₁₂+Na 861.4201. Found: for B2014 861.4178, for B2015 861.4160.



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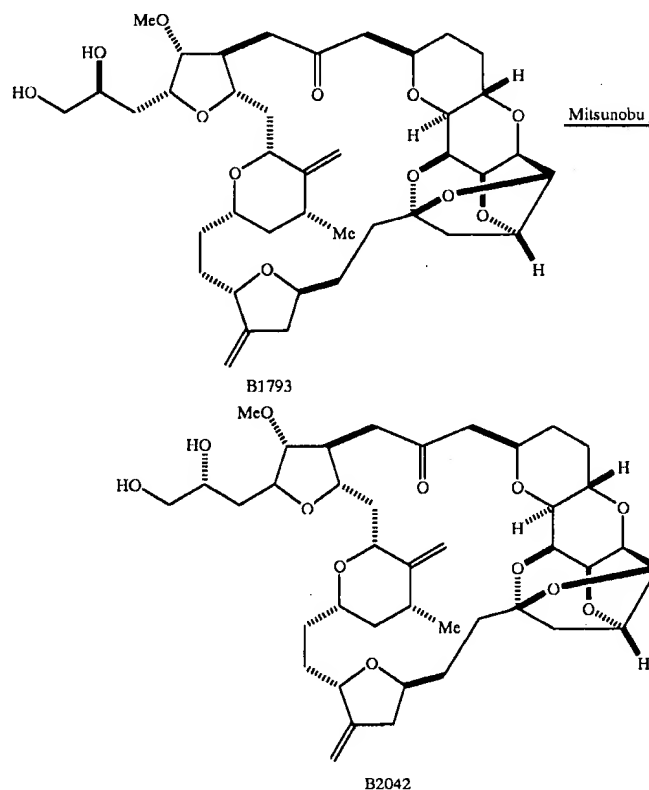
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General A mixture of B1793 (1 mg, 1.37 micromol), Et₃N (10 microl, 72 micromol) and ArNCO (2 to 4 equiv.) in CH₂Cl₂ (0.2 mL) was stirred at rt for 4 h to overnight until the reaction was judged to be complete by TLC. The reaction mixture was diluted with saturated NaHCO₃ (3 mL),

B1990 (1.1 mg, 92%) HRMS (FAB): calcd for C₄₇H₆₂ClNO₁₃+Na 906.3807. Found: 906.3826.

B1992 (1.0 mg, 83%) HRMS (FAB): calcd for C₄₈H₆₅NO₁₄+Na 902.4303. Found: 902.4269.



extracted with CH₂Cl₂ (3×) and EtOAc (2×), dried over Na₂SO₄ and purified by preparative TLC (5% MeOH—CH₂Cl₂) to afford the products:

B1984 (1.0 mg, 86%) HRMS (FAB): calcd for C₄₇H₆₃NO₁₃+Na 872.4197. Found: 872.4214.

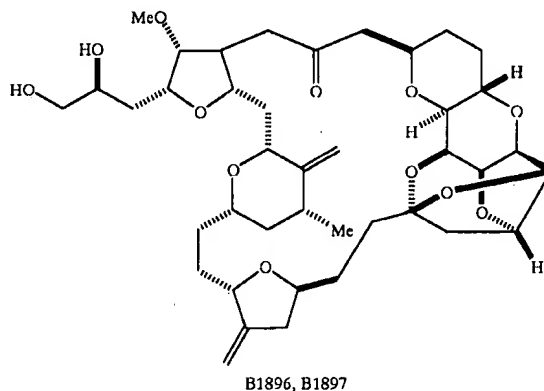
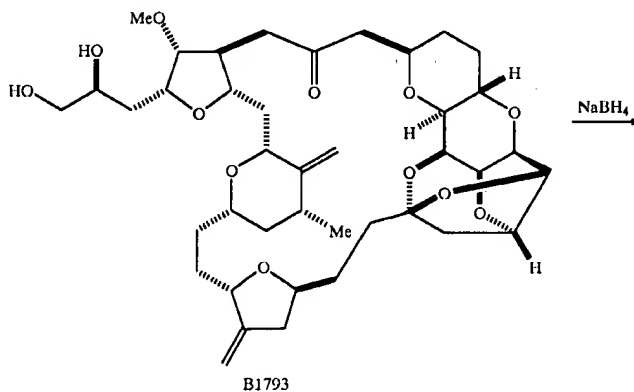
B2042 DEAD (0.4 M in ether, 50 μL, 19 μmol) was added to a solution of B1793 (2.0 mg, 2.7 μmol), triphenylphosphine (5 mg, 19 μmol), 4-nitrobenzoic acid (3.2 mg, 19 μmol) and Et₂O (500 μL) at rt. After 22 h, the reaction mixture was loaded directly onto a preparative TLC plate and eluted with 60% EtOAc-hexanes to give the intermedi-

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ate diester (3.0 mg). This material was taken up in MeOH (300 μ L) and treated with K_2CO_3 (approximately 1 mg). After stirring at rt for 30 min, the reaction mixture was diluted with brine and extracted with CH_2Cl_2 (5 \times). The combined extracts were dried over Na_2SO_4 , concentrated and purified by preparative TLC (5% MeOH—EtOAc) to afford B2042 (1.2 mg, 60% for two steps). HRMS (FAB): calcd for $C_{40}H_{58}O_{12}+Na$ 753.3826. Found: 753.3810.

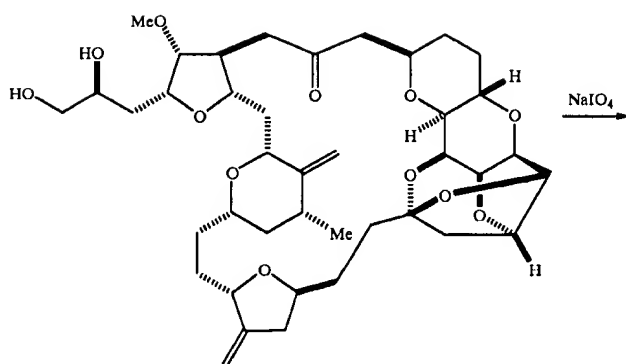
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B1896 and B1897 $NaBH_4$ (3 mg, 0.08 mmol) was added to a solution of B1793 (2.30 mg, 3.15 μ mol) in 1:1 CH_2Cl_2 —MeOH (0.2 mL) at rt. Concentration of the reaction mixture and purification by preparative TLC (8% MeOH—EtOAc) provided B1896 (0.80 mg, 35%) and B1897 (2:1 mixture, 0.15 mg, 6.5%). HRMS (FAB) for B1896: calcd for $C_{40}H_{60}O_{12}+Na$ 755.3983. Found: 753.3969.

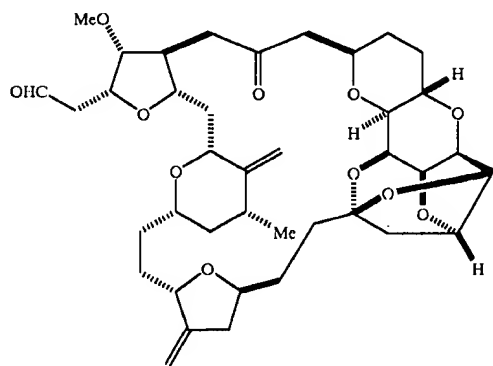


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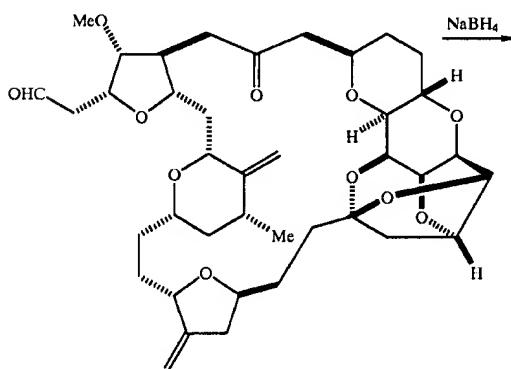


B1793

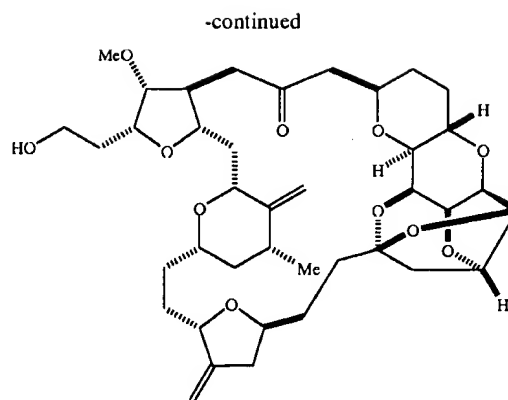


B1918

B1918 A mixture of B1793 (2.0 mg, 2.74 μ mol), NaIO₄ (35 mg, 0.16 mmol), MeOH (0.8 mL) and H₂O (0.2 mL) was stirred at rt for 40 min. The reaction mixture was diluted with H₂O (1 mL), extracted with CH₂Cl₂ (6 \times), dried over Na₂SO₄, concentrated and purified by column chromatography (5% MeOH—CH₂Cl₂) to give B1918 (1.9 mg, 100%).



B1918

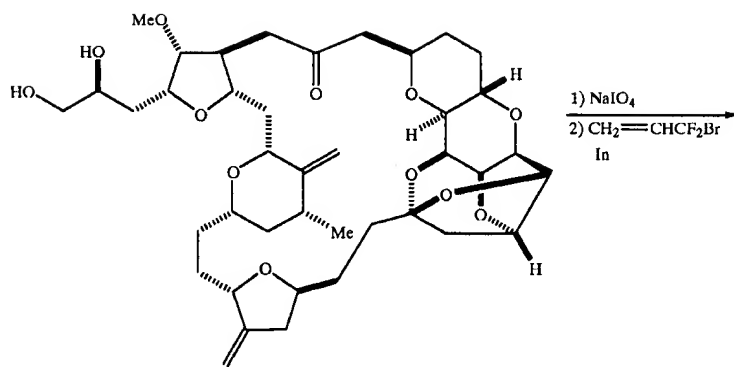


B2037

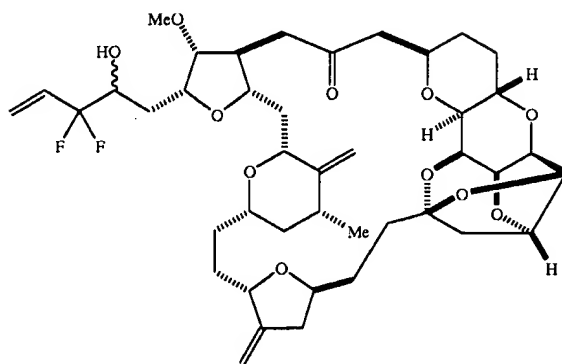
B2037 A 0.034 M solution of NaBH₄ (0.1 mL, 3.4 μ mol) in EtOH was added portion-wise to a solution of B1918 (1.9 mg, 2.72 μ mol) in MeOH (0.8 mL) and CH₂Cl₂ (0.2 mL) at -78° C. to rt until the reaction was judged to be complete by TLC. The reaction was quenched with saturated aqueous NH₄Cl (2 mL) at -78° C., warmed to rt, extracted with CH₂Cl₂ (6 \times), dried over Na₂SO₄ and purified by preparative TLC (5% MeOH—CH₂Cl₂) to afford B2037 (1.7 mg, 89%). HRMS (FAB): calcd for C₃₉H₅₆O₁₁+Na 723.3720. Found: 723.3749.

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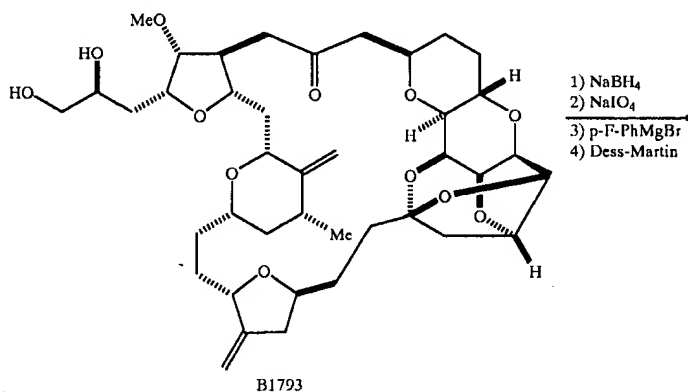
B1793



B2035

B2035 NaIO_4 (35 mg, 0.16 mmol) was added to a solution of B1793 (1.7 mg, 0.0023 mmol), MeOH (800 μL) and H_2O (200 μL) and after 15 min, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (5 \times). The organic extracts were dried over Na_2SO_4 , concentrated and the intermediate aldehyde was immediately dissolved in DMF (300 μL). 3-Bromo-3,3-difluoropropene (3 μL , 0.023 mmol) and indium powder (3 mg, 0.23 mmol) were added and after 24

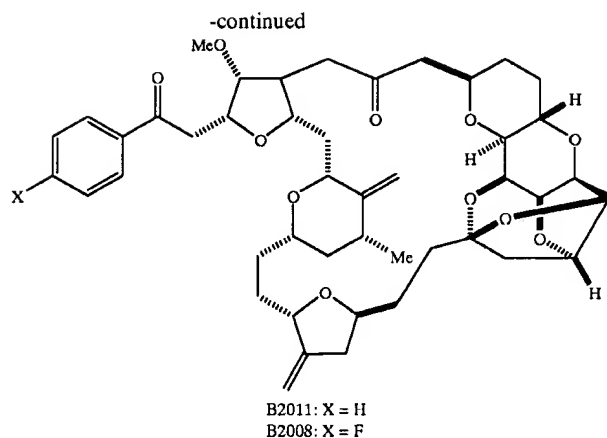
h additional 3-bromo-3,3-difluoropropene (1 μL , 0.008 mmol) was added. After 18 h, H_2O was added the mixture was extracted with EtOAc (3 \times). The combined organic extracts were washed successively with H_2O and brine, dried over Na_2SO_4 , concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide B2035 (0.74 mg, 41% for 2 steps) as a mixture of isomers. HRMS (FAB): calcd for $\text{C}_{42}\text{H}_{58}\text{F}_2\text{O}_{11}+\text{Na}$ 799.3845. Found: 799.3883.



B1793

67

68

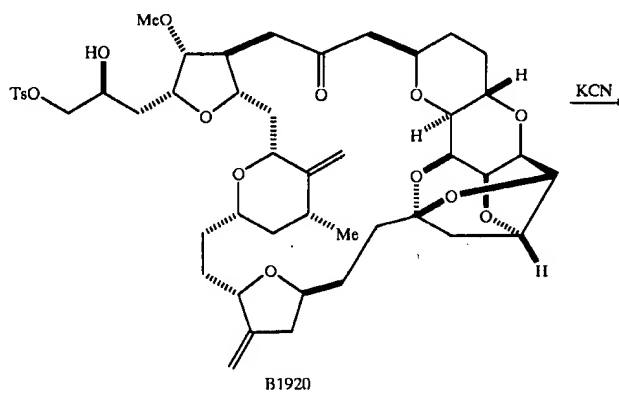


B2008, B2011 NaBH_4 (2 mg, 0.05 mmol) was added to a solution of B1793 (2.2 mg, 0.003 mmol) in 1:1 CH_2Cl_2 — MeOH (200 μL) at rt. After 15 min saturated aqueous NH_4Cl and H_2O were added, and the mixture was extracted with CH_2Cl_2 (6 \times) and EtOAc (2 \times). The combined organic extracts were dried over Na_2SO_4 , concentrated and purified by column chromatography (10% MeOH — EtOAc) to provide an intermediate triol, which was dissolved in MeOH (800 μL) and H_2O (200 μL). NaIO_4 (35 mg, 0.16 mmol) was added and after 20 min, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (6 \times). The organic extracts were dried over Na_2SO_4 , concentrated and the intermediate aldehyde was immediately dissolved in THF (500 μL). 4-Fluorophenylmagnesium bromide (2M in Et_2O , 12 μL , 0.024 mmol) was added and after 20 min the reaction was quenched with saturated aqueous NH_4Cl . The mixture was extracted with CH_2Cl_2 (6 \times) and the combined organic extracts were dried over Na_2SO_4 and concentrated. Purifi-

cation by preparative TLC (EtOAc) provided the desired product as a mixture of 4 isomers (1.32 mg, 55% for 3 steps).

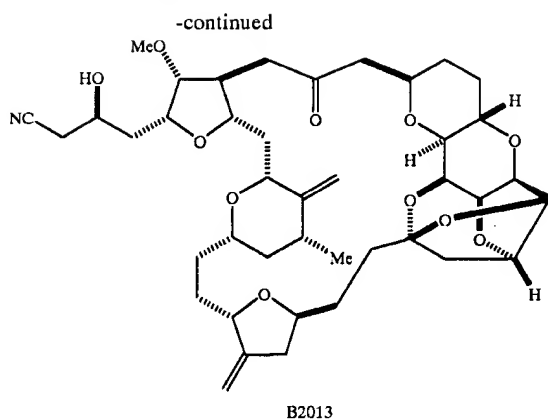
Dess-Martin periodinane (~3 mg, 0.007 mmol) was added to a solution of the above product (0.95 mg, 0.0012 mmol) in CH_2Cl_2 (300 μL) and the mixture was stirred at rt for 20 min. Additional Dess-Martin periodinane (~3 mg, 0.007 mmol) and CH_2Cl_2 (300 μL) were added and after another 40 min Et_2O , saturated aqueous NaHCO_3 (4 mL) and saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (1 mL) were added. The mixture was extracted with Et_2O (3 \times) and the combined extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (20% EtOAc —hexanes) to provide B2008 (0.58 mg, 61%). HRMS (FAB): calcd for $\text{C}_{45}\text{H}_{57}\text{FO}_{11}+\text{Na}$ 815.3783. Found: 815.3758.

B2011 In an analogous manner, B1793 (1.9 mg, 0.003 mmol) was converted to B2011 (0.87 mg, 42% for 4 steps). HRMS (FAB): calcd for $\text{C}_{45}\text{H}_{58}\text{O}_{11}+\text{Na}$ 797.3877. Found: 797.3877.



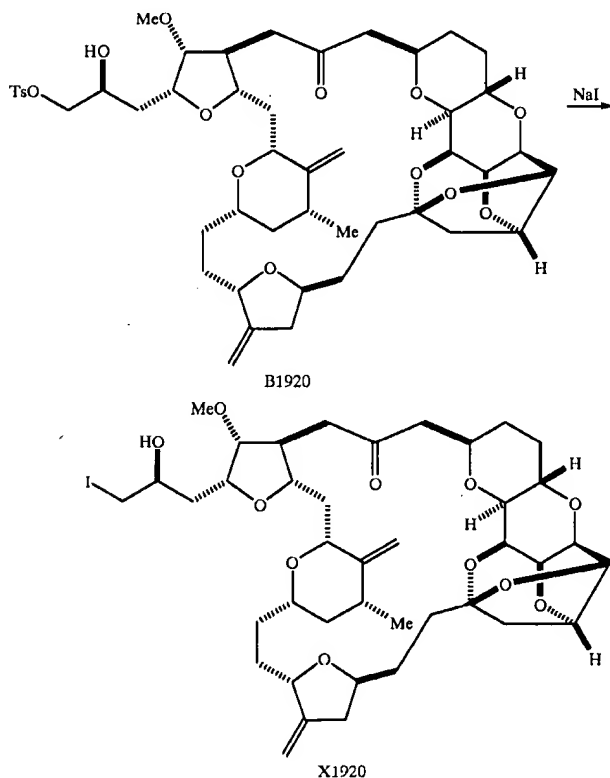
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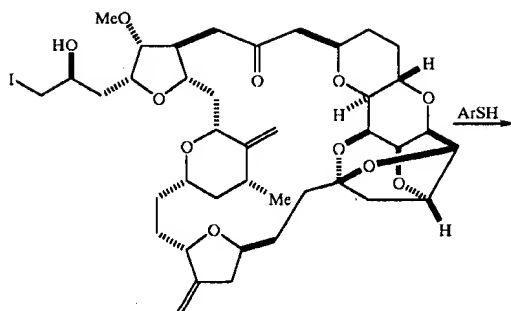


B2013 A solution of B1920 (1.4 mg, 0.0016 mmol), KCN (1 mg, 0.016 mmol) and DMSO (500 μ L) was heated at 60° C. for 8 h. After cooling to rt, H₂O was added and the mixture was extracted with EtOAc (3 \times). The combined organic extracts were washed successively with H₂O and brine, dried over Na₂SO₄, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide B2013 (0.78 mg, 67%). HRMS (FAB): calcd for C₄₁H₅₇NO₁₁+Na 762.3829. Found: 762.3848.

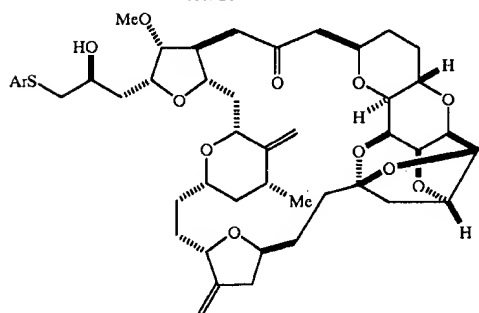
X1920 A mixture of B1920 (1.3 mg, 1.47 μ mol), NaI (30 mg, excess) and acetone (1 mL) was stirred at 60° C. for 3.5 h. After cooling to rt, the reaction mixture was diluted with saturated aqueous NaHCO₃ (3 mL), extracted with CH₂Cl₂ (5 \times) and EtOAc, dried over Na₂SO₄ and purified by column chromatography (50% EtOAc-CH₂Cl₂ to 80% EtOAc-hexanes) to give the iodide X1920 (1.3 mg, 100%).



71



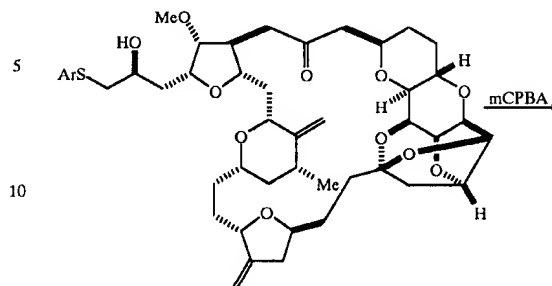
X1920



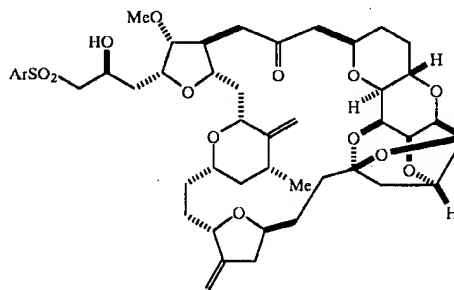
B1998: Ar = p-Cl-Ph
 B2010: Ar = p-MeO-Ph
 B2019: Ar = 2-imidazole

72

B2019 (1.1 mg gave 0.7 mg, 61%) MS (FAB): M+Na



B1998: Ar = p-Cl-Ph
 B2010: Ar = p-MeO-Ph



B2016: Ar = p-Cl-Ph
 B2030: Ar = p-MeO-Ph

General A mixture of iodide X1920 (1.0 equiv.), $i\text{Pr}_2\text{EtN}$ (11 to 22 equiv.), ArSH (9 to 46 equiv.) and DMF (0.3 mL) was stirred at rt until the reaction was judged to be complete by TLC (typically 24 to 48 h). The reaction mixture was diluted with saturated aqueous NaHCO_3 (2 mL), extracted with CH_2Cl_2 and EtOAc, dried over Na_2SO_4 and purified by preparative TLC (80% EtOAc-hexanes or 5% MeOH— CH_2Cl_2) to afford the sulfide products:

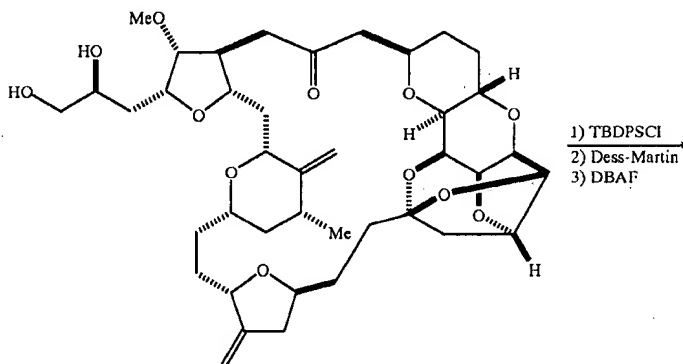
B1998 (1.3 mg gave 1.1 mg, 85%) HRMS (FAB): calcd for $\text{C}_{46}\text{H}_{61}\text{ClO}_{11}\text{S}+\text{Na}$ 897.3521. Found: 897.3533.

B2010 (1.1 mg gave 0.7 mg, 59%). HRMS (FAB): calcd for $\text{C}_{47}\text{H}_{64}\text{O}_{12}\text{S}+\text{Na}$ 875.4016. Found: 875.4057.

General A 0.01 M solution of mCPBA (1.2 equiv.) in CH_2Cl_2 was added to a solution of a sulfide (1.0 equiv.) in CH_2Cl_2 (0.5 mL) at 0°C . for 30 min. The reaction mixture was diluted with saturated NaHCO_3 (2 mL), extracted with CH_2Cl_2 and EtOAc, dried over Na_2SO_4 and purified by preparative TLC (80% EtOAc-hexanes or EtOAc) to afford the products:

B2016 (0.9 mg gave 0.7 mg, 74%)

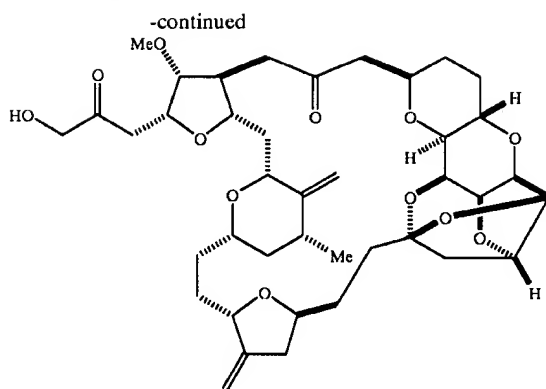
B2030 (1.0 mg gave 0.6 mg, 61%) HRMS (FAB): calcd for $\text{C}_{47}\text{H}_{64}\text{O}_{14}\text{S}+\text{Na}$ 907.3914. Found: 907.3950.



B1793

73

74

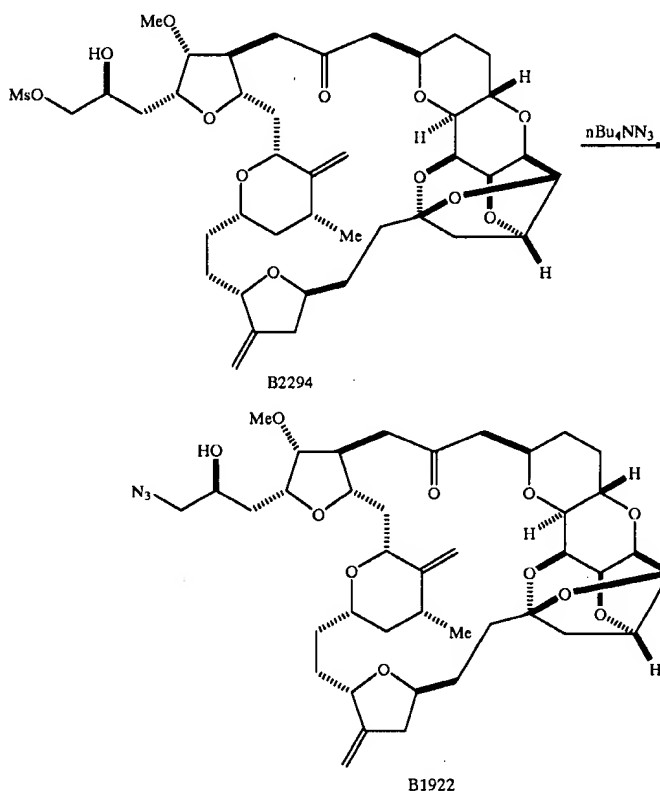


B1934 TBPSCl (3.0 μ L, 12 μ mol) was added to a solution of B1793 (1.3 mg, 1.78 μ mol), imidazole (10 mg, 166 μ mol) and DMF (0.10 mL) at rt. After stirring for 1 h, the reaction mixture was diluted with saturated aqueous NaHCO₃ (2 mL), extracted with CH₂Cl₂ (3x) and EtOAc (2x), dried over Na₂SO₄ and purified by preparative TLC (5% MeOH—CH₂Cl₂) to give the intermediate silyl ether (1.3 mg, 77%).

This material was dissolved in CH₂Cl₂ (0.5 mL) and treated with Dess-Martin periodinane (10 mg, 24 μ mol) for 1.5 h at rt, diluted with Et₂O and filtered through Celite. The filtrate was concentrated and purified by preparative TLC

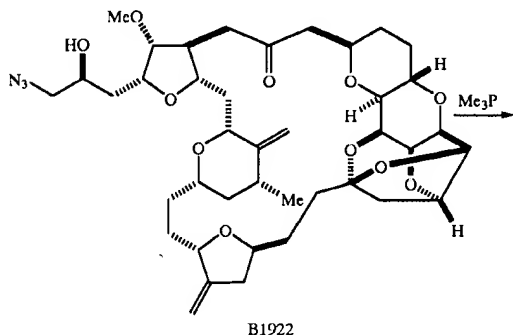
(50% EtOAc-hexanes) to afford the diketone intermediate (1.0 mg, 77%), which was dissolved in THF (0.5 mL) and treated with 0.02 M TBAF containing 0.01 M imidazole hydrochloride (THF solution, 75 μ L, 1.5 μ mol) at rt for 15 min. The reaction mixture was eluted through a SiO₂ column (50% EtOAc-hexanes to 5% MeOH—CH₂Cl₂) and the desired product was further purified by preparative TLC (5% MeOH—CH₂Cl₂) to afford B1934 (0.75 mg, 100%). HRMS (FAB): calcd for C₄₀H₅₆O₁₂+Na 751.3669. Found: 751.3690.

Synthesis of B1939:



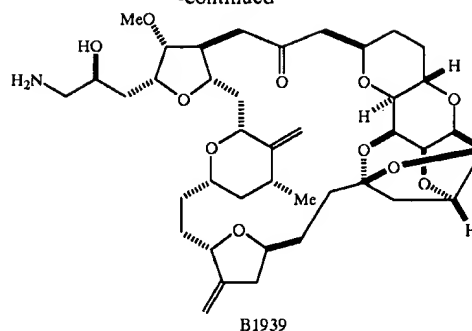
75

B1922 Tetra-*n*-butylammonium azide (0.2 M in DMF, 0.5 mL, 0.10 mmol) was added to a solution of mesylate B2294 (21.4 mg, 0.026 mmol) in DMF (2 mL) at rt. After stirring at 83° C. for 3.5 h, the reaction mixture was cooled to rt, diluted with toluene, concentrated and purified by preparative TLC (80% ethyl acetate-hexanes) to furnish B1922 (18 mg, 92%).



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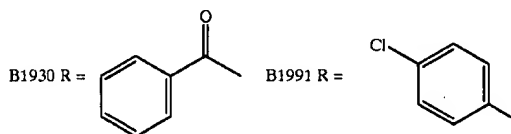
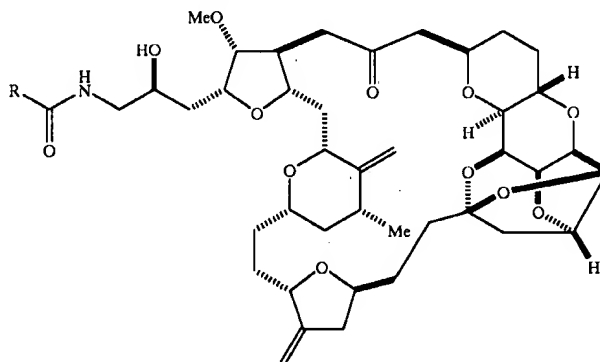
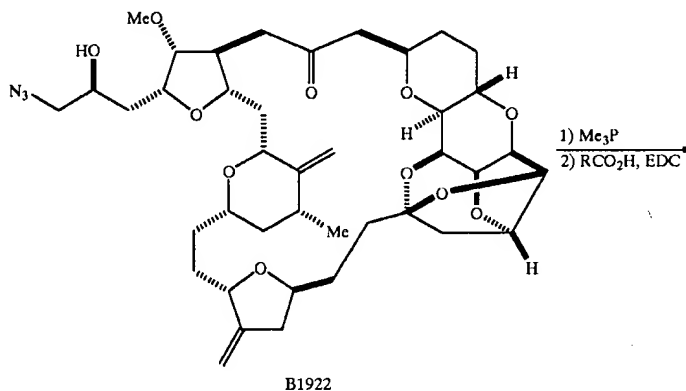
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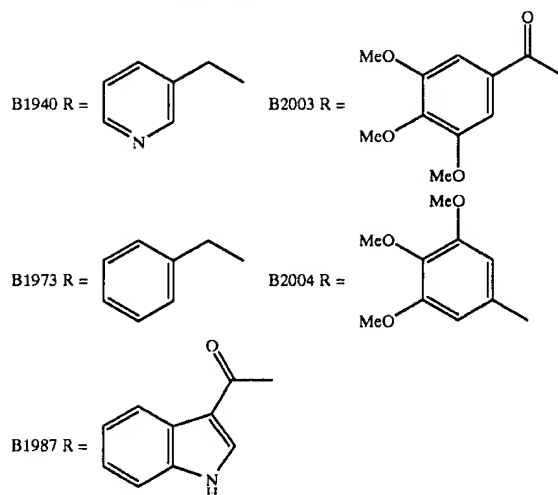


B1939 Me₃P (1 M in THF) and H₂O (0.8 mL) were sequentially added to a solution of azide B1922 (24.6 mg, 0.032 mmol) in THF (3.2 mL) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated and purified by flash chromatography [step gradient, 10% MeOH—EtOAc followed by MeOH—EtOAc-30% aqueous NH₄OH (9:86:5)] to provide the desired primary amine (23.3 mg), which by ¹H-NMR contained ~1% trimethylphosphine oxide. Lyophilization from benzene and standing under high vacuum for 2 d furnished B1939 (20.3 mg, 87%).

Synthesis of Representative B1939 Analogs:

B1930, B1940, B1973, B1987, B1988, B1991, B2003, B2004





B1930 Me₃P (1 M in THF, 13 μ L, 0.013 mmol) was added to a solution of B1922 (1.6 mg, 2.1 μ mol), THF (400 μ L) and H₂O (100 μ L) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated, and azeotropically dried with toluene (2x) to give the crude amine which was used directly in the next step.

EDC (0.06 M in CH₂Cl₂, 100 μ L, 11 μ mol) was added to a solution of the crude amine, benzoylformic acid (0.8 mg, 5.3 μ mol) and CH₂Cl₂ (200 μ L) at rt. After 30 min, the reaction was quenched with a 1:4 mixture of saturated aqueous NaHCO₃-brine and extracted with CH₂Cl₂ (5x). The combined extracts were dried over Na₂SO₄ and concentrated and purified by preparative TLC (EtOAc) to afford B1930 (1.5 mg, 83% for two steps). HRMS (FAB): calcd for C₄₈H₆₃NO₁₃+Na 884.4166.

B1940 Using the procedure described above for B1930, B1922 was reduced, coupled with 3-pyridylacetic acid hydrochloride and purified by preparative TLC [(MeOH—EtOAc—30% aqueous NH₄OH (9:86:5))] to afford B1940 (0.8 mg, 67% for two steps). HRMS (FAB): calcd for C₄₇H₆₄N₂O₁₂+Na 871.4357. Found: 871.4362.

B1973 Using the procedure described above, B1922 (0.9 mg, 1.2 μ mol) was reduced, coupled with phenylacetic acid and purified by preparative TLC (5% MeOH—EtOAc) to

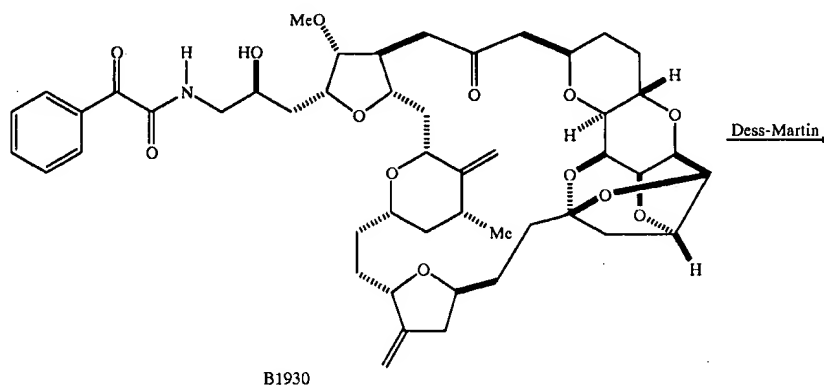
afford B1973 (0.44 mg, 44% for two steps). HRMS (FAB): calcd for C₄₈H₆₅NO₁₂+Na 870.4404. Found: 870.4447.

B1987 Using the procedure described above, B1922 (0.9 mg, 1.2 μ mol) was reduced, coupled with 3-indoleglyoxylic acid and purified by preparative TLC (3% MeOH—EtOAc) to afford B1987 (0.8 mg, 75% for two steps). HRMS (FAB): calcd for C₅₀H₆₄N₂O₁₂+Na 923.4306. Found: 923.4338.

B1991 Using the procedure described above, B1922 (1.0 mg, 1.3 μ mol) was reduced, coupled with 4-chlorobenzoic acid and purified by preparative TLC (3% MeOH—EtOAc) to afford B1991 (0.8 mg, 70% for two steps). HRMS (FAB): calcd for C₄₇H₆₂ClNO₁₂+Na 890.3858. Found: 890.3843.

B2003 Using the procedure described above, B1922 (1.0 mg, 1.3 μ mol) was reduced, coupled with 3,4,5-trimethoxybenzoylformic acid and purified by preparative TLC (EtOAc) to afford B2003 (0.7 mg, 56% for two steps). HRMS (FAB): calcd for C₅₁H₆₉NO₁₆+Na 974.4514. Found: 974.4525.

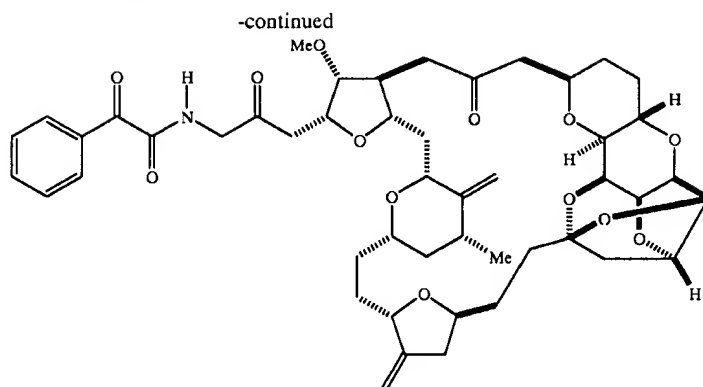
B2004 Using the procedure described above, B1922 (1.0 mg, 1.3 μ mol) was reduced, coupled with 3,4,5-trimethoxybenzoic acid and purified by preparative TLC (5% MeOH—EtOAc) to afford B2004 (0.7 mg, 58% for two steps). HRMS (FAB): calcd for C₄₉H₆₅NO₁₃+Na 946.4565. Found: 946.4599.



B1930

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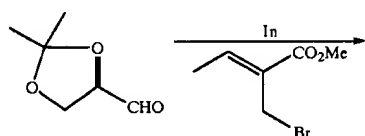
80



B1988

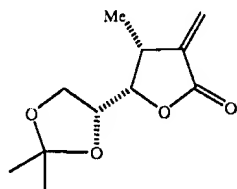
B1988 Dess-Martin periodinane (1 mg, 2.3 μ mol) was added to a solution of B1930 (0.80 mg, 0.93 μ mol) in CH_2Cl_2 (500 μL) at rt. After 1 h, the reaction was diluted with Et_2O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous NaHCO_3 — $\text{Na}_2\text{S}_2\text{O}_3$ and brine, dried over Na_2SO_4 , concentrated and purified by preparative TLC (80% EtOAc-hexanes) to afford B1988 (0.45 mg, 56%). HRMS (FAB): calcd for $\text{C}_{48}\text{H}_{61}\text{NO}_{13}+\text{Na}$ 882.4041. Found: 884.4012.

Synthesis of B2090:

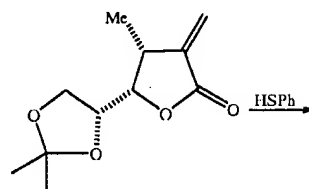


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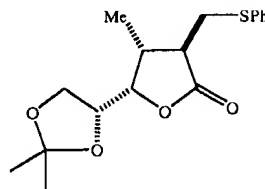
102



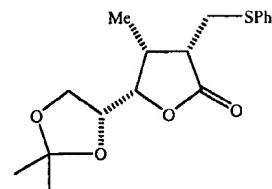
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103



104

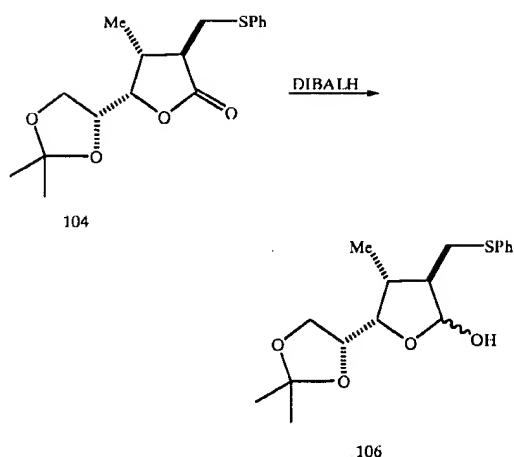


105

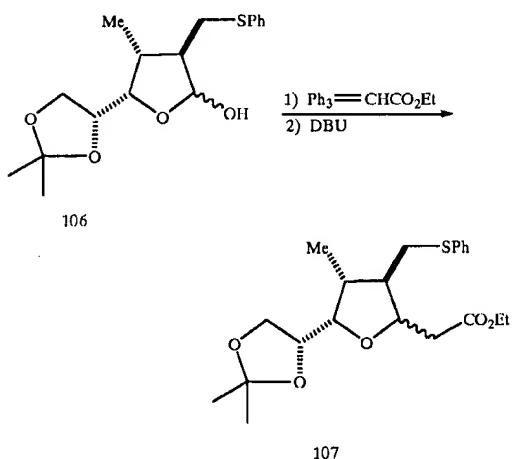
Compound 103 Indium powder (1.35 g, 11.8 mmol) was added to a solution of 102 (3.38 g, 17.6 mmol) in DMF (20 mL) at rt. After stirring for 30 min, the reaction mixture was cooled to 0° C. Neat aldehyde 101 (3.72 g, 28.6 mmol) was then added and the mixture was stirred overnight while allowing the temperature to warm to rt. The reaction mixture was recooled to 0° C. and then quenched carefully with saturated aqueous NH_4Cl (100 mL). After stirring for 30 min, the resulting mixture was extracted with Et_2O (3 \times), dried over Na_2SO_4 , concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to give pure crystalline 103 (2.20 g, 59%).

Compound 104 Et_3N (72 μL , 0.51 μ mol) was added to a solution of 103 (1.09 g, 5.13 mmol) and thiophenol (0.63 mL, 7.16 mmol) in CH_2Cl_2 and the resulting mixture was stirred at 0° C. for 1 h. Filtration through SiO_2 gave a mixture of 104 and 105, which after MPLC (15% to 20% EtOAc-hexanes) afforded 104 (0.53 g, 32%) and 105 (0.92 g, 56%).

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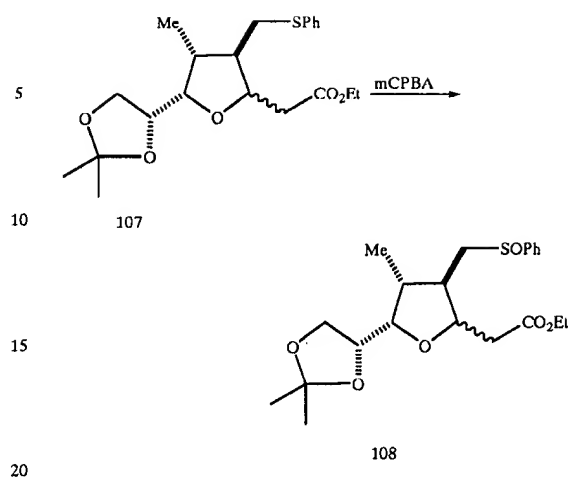


Compound 106 DIBALH (1 M in toluene, 3.28 mL, 3.28 mmol) was added to a solution of 104 (0.53 g, 1.64 mmol) in toluene (10 mL) at -78°C . and the mixture was stirred at -78°C . for 10 min. The reaction was quenched by careful addition of MeOH (0.40 mL, 9.84 mmol) and H_2O (0.17 mL, 9.84 mmol), warmed to rt and stirred for 20 min. The white suspension was filtered through a mixture of Celite and SiO_2 with 1:1 CH_2Cl_2 — Et_2O and concentrated to give 106 (0.53 g, 100%) as an oil.

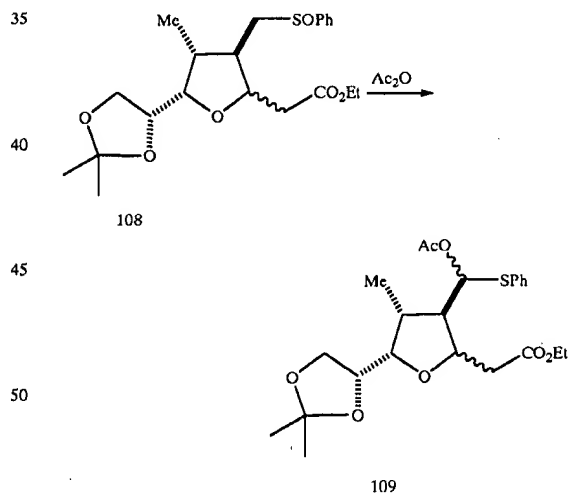


Compound 107 A mixture of 106 (0.53 g, 1.64 mmol) and ethyl (triphenylphosphoranylidene)acetate (1.15 g, 3.29 mmol) in toluene (10 mL) was heated to 80°C . for 15 h. The mixture was cooled to rt and DBU (25 μL , 0.16 mmol) was introduced. The mixture was heated to 80°C . for 1.5 h, cooled to rt, concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to give 107 (0.54 g, 83%) as an oil (3:1 ratio of α : β isomers).

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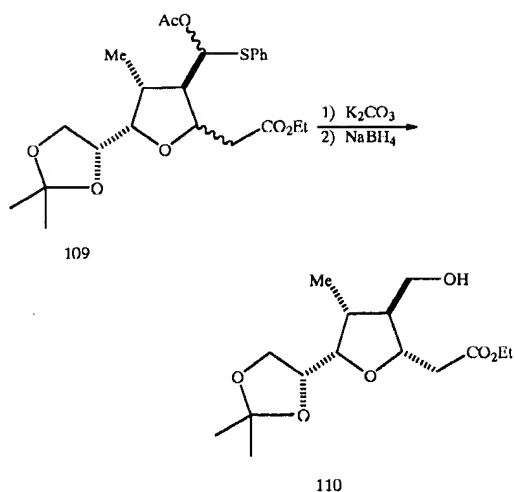


Compound 108 A solution of mCPBA (~55%, 450 mg in 4.5 mL CH_2Cl_2 , 1.44 mmol) was added to a solution of 107 (0.54 g, 1.36 mmol) in CH_2Cl_2 (10 mL) at -78°C . The reaction mixture was diluted with saturated aqueous NaHCO_3 (50 mL), H_2O (10 mL), and Et_2O (60 mL) and then warmed to rt. The separated aqueous layer was extracted with EtOAc (4 \times) and the combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (50% EtOAc-hexanes) to give 108 (0.51 g, 92%) as an oil.

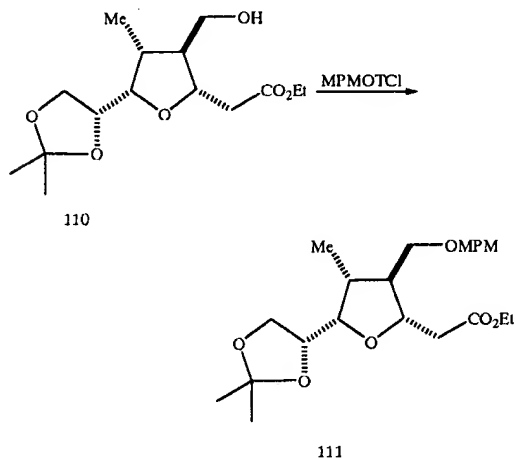


Compound 109 A mixture of 108 (0.51 g, 1.24 mmol) and NaOAc (1.00 g, 12.4 mmol) in Ac_2O (10 mL) was stirred at 140°C . for 12 h, cooled to rt and then concentrated. The residue was partitioned between saturated aqueous NaHCO_3 (20 mL) and Et_2O (30 mL), and stirred vigorously at rt for 30 min. The separated aqueous layer was extracted with Et_2O (2 \times), and the combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (5% to 15% EtOAc-hexanes) to give 109 (0.41 g, 73%) as an oil.

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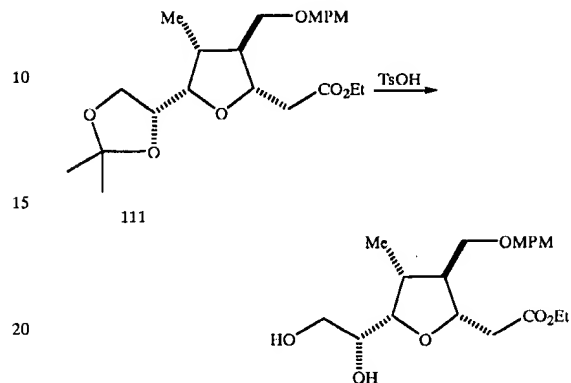
Compound 110 A mixture of 109 (0.41 g, 0.91 mmol) and K_2CO_3 (44.3 mg, 0.32 mmol) in EtOH (5 mL) was heated to 60–70° C. for 1 d. After cooling to rt, the reaction mixture was concentrated and eluted through a SiO_2 column (10% to 20% EtOAc-hexanes) to give the partially purified aldehyde intermediate. This material was dissolved in EtOH (2.5 mL), treated with $NaBH_4$ (50 mg, 1.32 mmol) and stirred at rt for 30 min. The mixture was concentrated and purified by column chromatography (40% EtOAc-hexanes) to give 110 (181 mg, 66%).



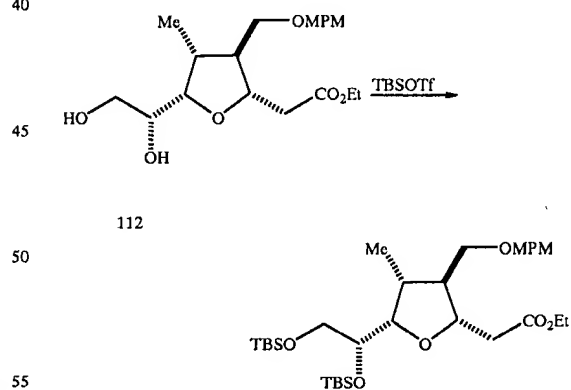
Compound 111 $BF_3 \cdot OEt_2$ (0.05 M in CH_2Cl_2 , 175 μL , 8.75 μmol) was added to a solution of 110 (181 mg, 0.60 mmol) and p-methoxybenzyl 2,2,2-trichloroacetimidate (0.50 mL, 1.80 mmol) in CH_2Cl_2 (5 mL) at 0° C. The resulting mixture was stirred for 1.5 h at 0° C. and for 2 h at rt until the reaction was complete. The mixture was

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quenched with saturated aqueous $NaHCO_3$ (25 mL) and extracted with Et_2O (5 \times). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (CH_2Cl_2 and then 20% EtOAc-hexanes) to give semi-pure 111 (0.37 g, >100%) as an oil.

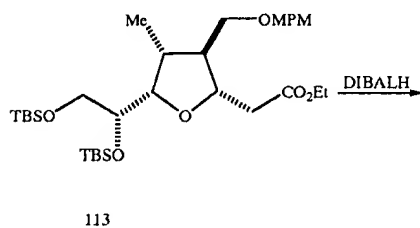


Compound 112 A mixture of 111 (0.37 g, max.=0.60 mmol) and $TsOH \cdot H_2O$ (36 mg) in EtOH (5 mL) was stirred initially at rt overnight and then at 60° C. for 1 h. Additional $TsOH \cdot H_2O$ (31 mg) was added at rt and the reaction mixture was stirred for 1 h at rt. The mixture was then concentrated, quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc (5 \times). The combined organic phases were dried over Na_2SO_4 , concentrated and purified by column chromatography (20% to 50% EtOAc-hexanes and then 5% MeOH- CH_2Cl_2) to give 112 (121 mg, 53%) as an oil along with recovered 111 (49 mg, 21%).



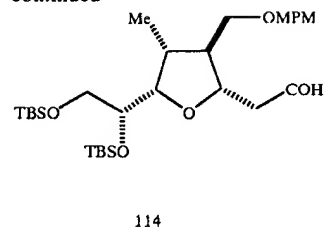
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Compound 113 TBSOTf (250 μ L, 1.09 mmol) was added to a solution of 112 (121 mg, 0.32 mmol) and Et₃N (176 μ L, 1.26 mmol) in CH₂Cl₂ at 0° C. and the resulting mixture was stirred for 25 min. The reaction was quenched with saturated aqueous NaHCO₃ (15 mL) and the separated aqueous layer was extracted with ether (3 \times). The combined organic phases were dried over Na₂SO₄, concentrated and purified by column chromatography (5% to 10% EtOAc/hexanes) to give 113 (165 mg, 85%) as an oil.

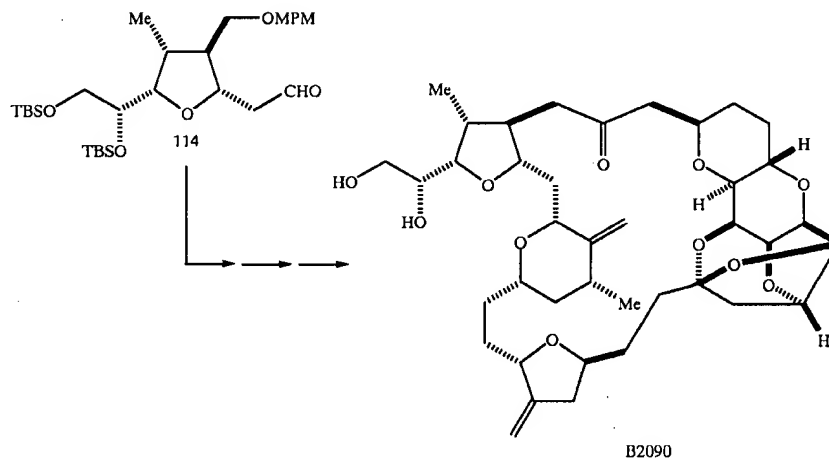


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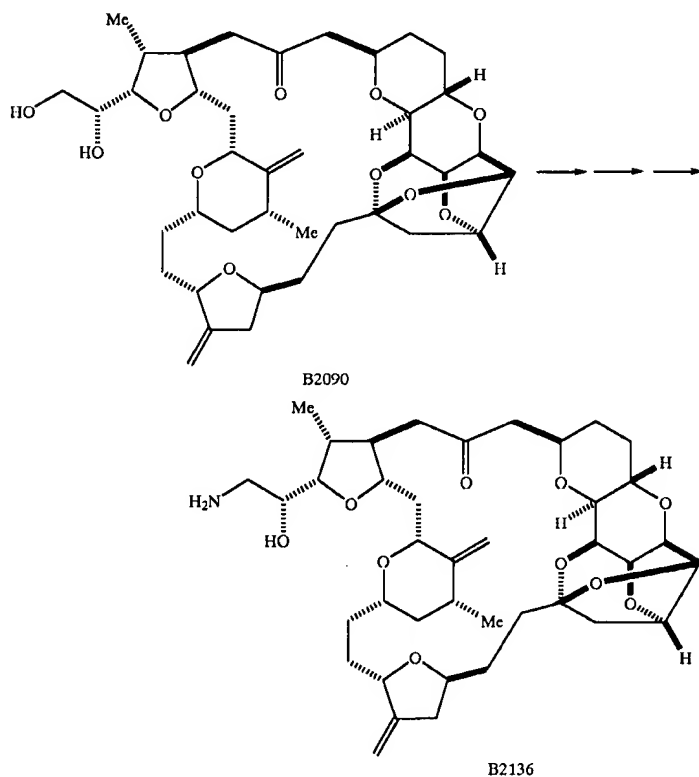


Compound 114 DIBALH (1 M in toluene, 0.54 mL, 0.54 mmol) was added to a solution of 113 (165 mg, 0.27 mmol) in toluene (5 mL) at -78° C. and the resulting mixture was stirred at -78° C. for 10 min. The reaction was quenched by careful addition of MeOH (65 μ L, 0.81 mmol) and H₂O (29 μ L, 0.81 mmol), warmed to rt and stirred for 25 min. The white suspension was filtered through Celite with 1:1 CH₂Cl₂—Et₂O. Concentration and purification by column chromatography (10% to 20% EtOAc-hexanes) gave 114 (153 mg, 100%) as an oil.



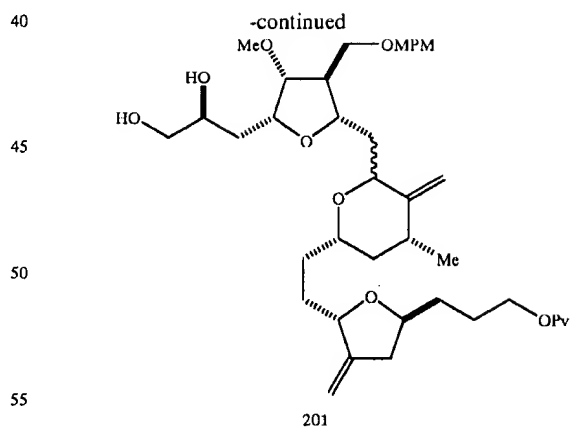
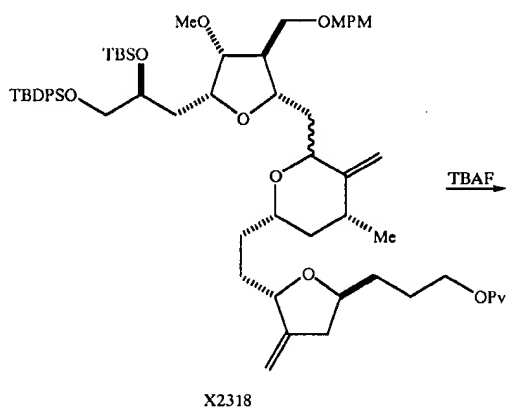
87

B2090 In a manner similar to that described in Scheme 6 for the synthesis of B1794, intermediate 114 was converted to B2090. HRMS (FAB): calcd for $C_{39}H_{56}O_{11}+Na$ 723.3720. Found: 723.3731.



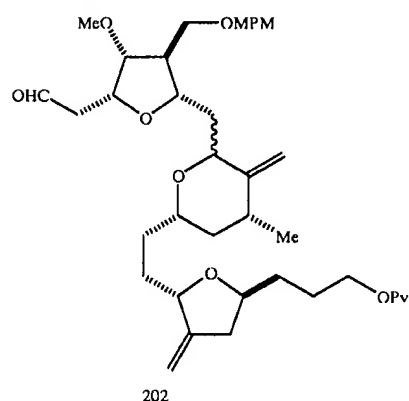
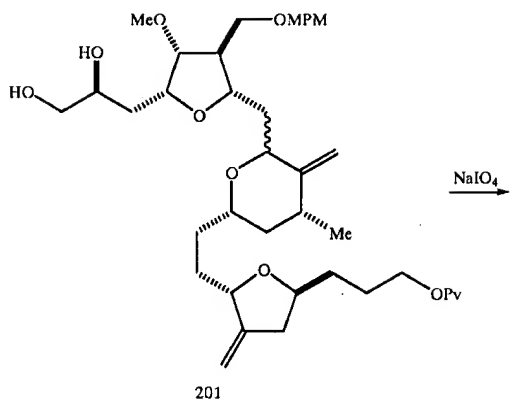
B2136 In a manner analogous to that of B1939, B2090 was converted to B2136. HRMS (FAB): calcd for $C_{39}H_{57}NO_{10}+Na$ 722.3880. Found: 722.3907.

Synthesis of B2039/B2043:

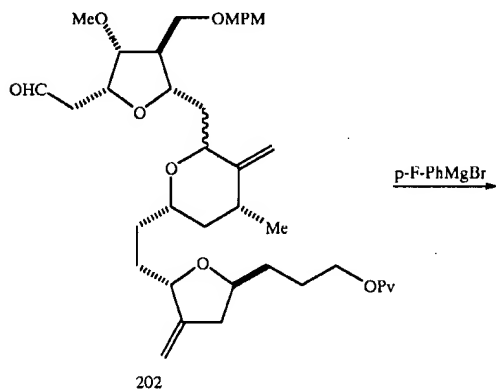


Diol 201 TBAF (1 M in THF, 383 μ L, 0.383 mmol) was added to a solution of X2318 (350-LS-218) (80.8 mg, 0.0765 mmol) in THF (7 mL) and stirred at rt for 16 h. After partial concentration, the residue was loaded directly onto a SiO_2 column packed using 30% EtOAc-hexanes. Gradient elution (30% EtOAc-hexanes to EtOAc) furnished diol 201 (49.7 mg, 92%).

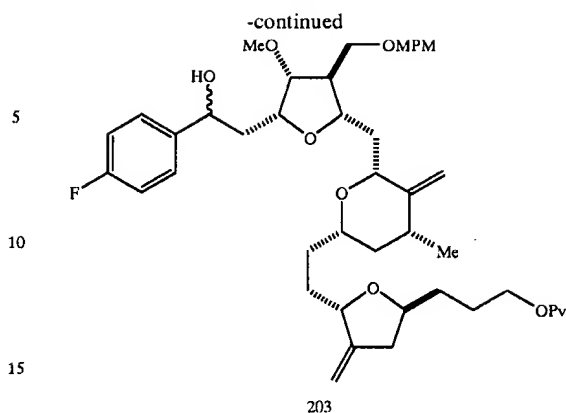
89



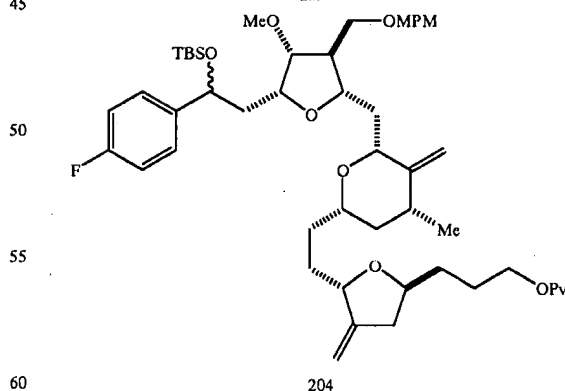
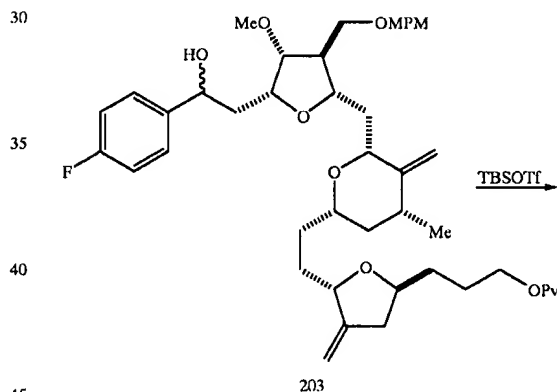
Aldehyde 202 A mixture of diol 201 (49.7 mg, 0.0707 mmol), NaIO₄ (100 mg, 0.47 mmol), MeOH (10 mL) and H₂O (2.5 mL) was stirred at rt for 30 min. H₂O was added and the mixture was extracted with CH₂Cl₂ (4×). The combined organic extracts were dried over Na₂SO₄, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide aldehyde 202 (41.7 mg, 88%).



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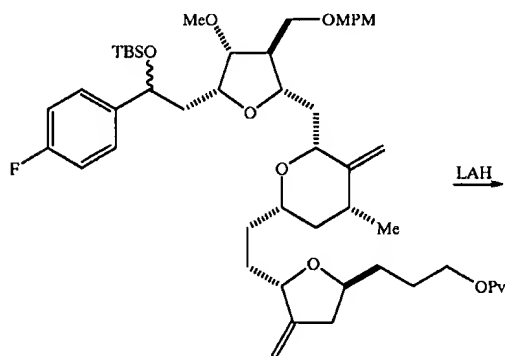
Alcohol 203 4-Fluorophenylmagnesium bromide (2 M in Et₂O, 155 μL, 0.31 mmol) was added to a solution of aldehyde 202 (41.7 mg, 0.062 mmol) in THF (6 mL). After 15 min at rt, the reaction was quenched with saturated aqueous NH₄Cl and extracted with CH₂Cl₂ (4×). The combined organic extracts were dried over Na₂SO₄, concentrated and purified by preparative TLC (40% EtOAc-hexanes) to provide alcohol 203 (32.4 mg, 68%) as a 1:1 mixture of C34 isomers. The minor undesired C27 isomer was separated at this stage and was also isolated as a 1:1 mixture of C34 isomers (8.4 mg, 18%).



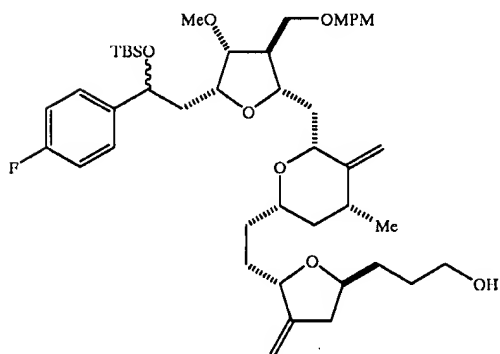
Ether 204 Et₃N (18 μL, 0.13 mmol) and TBSOTf (15 μL, 0.063 mmol) were added to a solution of alcohol 203 (32.4

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mg, 0.042 mmol) in CH_2Cl_2 (5 mL) at 0°C . After 20 min the reaction was quenched by the addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 \times). The combined organic extracts were dried over Na_2SO_4 , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide ether 204 (33.1 mg, 89%).

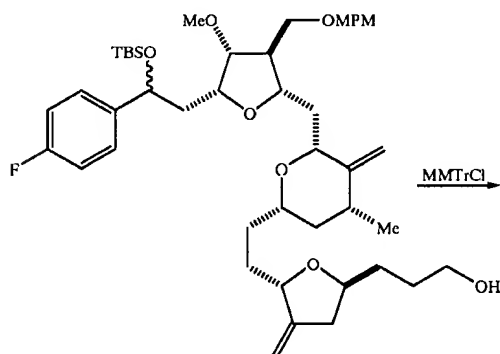


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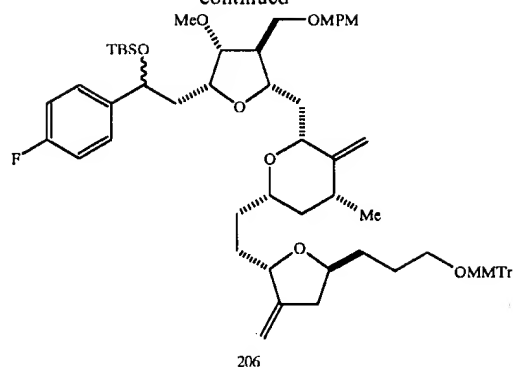
Alcohol 205 LAH (1 M in THF, 113 μL , 0.113 mmol) was added dropwise to a solution of ether 204 (33.1 mg, 0.0375 mmol) in Et_2O (10 mL) at 0°C . After 20 min, H_2O and 1 M NaOH were added and the mixture was stirred at rt for 10 min. Filtration through Celite, concentration and purification by column chromatography (40% EtOAc-hexanes) furnished alcohol 205 (28.4 mg, 95%).



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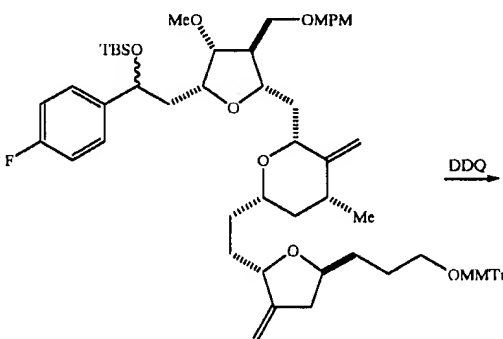
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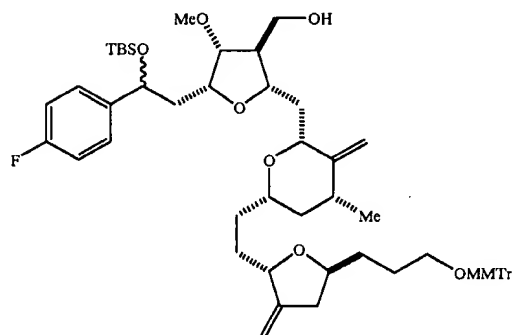


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Ether 206 Diisopropylethylamine (31 μL , 0.18 mmol) and MMTTrCl (22 mg, 0.071 mmol) were added to a solution of alcohol 205 (28.4 mg, 0.0356 mmol) in CH_2Cl_2 (4 mL) at 0°C . After 15 h at rt, H_2O was added and the mixture was extracted with CH_2Cl_2 (3 \times). The combined extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by preparative TLC (40% EtOAc-hexanes) to provide ether 206 as a ~1.5:1 mixture of C34 epimers (45 mg, quant), which contained a small amount of close-running impurities.



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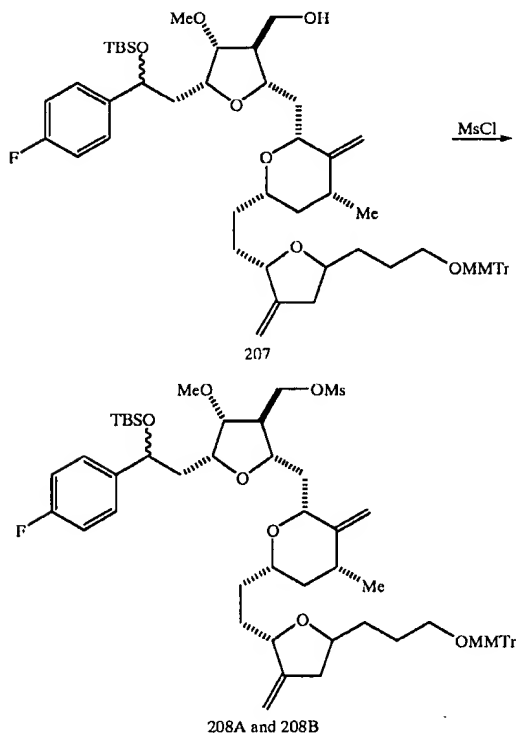


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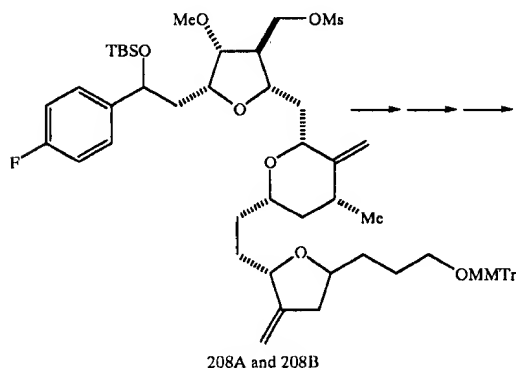
Alcohol 207 DDQ (40 mg, 0.18 mmol) was added to a solution of ether 206 (37 mg, 0.034 mmol) in CH_2Cl_2 (4 mL) and a 1:10 mixture of tBuOH :pH 7 phosphate buffer (2 mL) at 0°C . The mixture was stirred vigorously in the dark for 15 min. Three additional portions of DDQ (40 mg, 0.18 mmol) were added at 10 min intervals, then the reaction was diluted with saturated aqueous NaHCO_3 and extracted with CH_2Cl_2 (3 \times). The combined organic extracts were washed

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with brine, dried over Na_2SO_4 , concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide alcohol 207 (19.2 mg, 59%) as well as recovered ether 206 (9.7 mg, 26%).

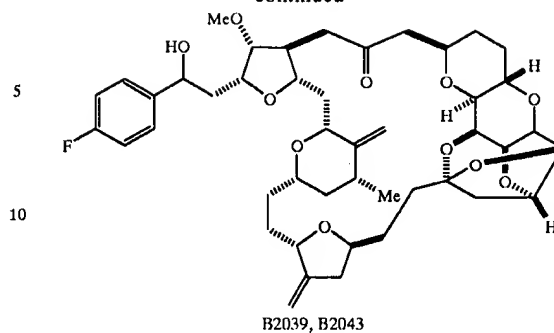


Mesylates 208A and 208B (Et_3N (19 μL , 0.13 mmol) and Ms_2O (10 mg, 0.056 mmol) were sequentially added to a solution of alcohol 207 (21.3 mg, 0.022 mmol) in CH_2Cl_2 (6 mL) at 0°C . After 30 min, saturated aqueous NaHCO_3 was added and the mixture was extracted with CH_2Cl_2 (3x). The combined extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide mesylates 208A (11.7 mg, 51%) and 208B (6.5 mg, 28%) as single C34 isomers.



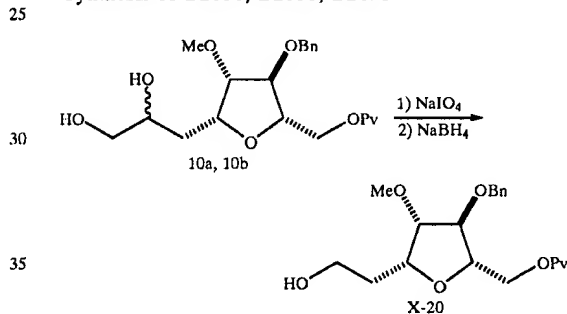
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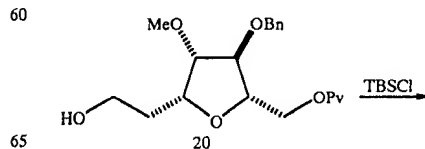
B2039 and B2043 in a manner similar to that described in Scheme 6 for the synthesis of B1794, both diastereomers 208A and 208B were independently converted to B2039 and B2043. HRMS (FAB): calcd for $\text{C}_{45}\text{H}_{55}\text{FO}_{11} + \text{Na}$ 817.3939. Found: for B2039 817.3896, B2043 817.3910.

Synthesis of B2086, B2088, B2091



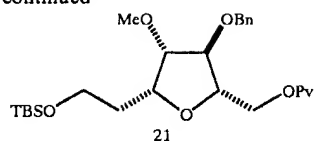
Alcohol X-20 (NaIO_4 (1.16 g, 5.4 mmol) was added to a solution of diols 10a, b (1.19 g, 3.0 mmol) in $\text{MeOH}-\text{H}_2\text{O}$ (4:1, 75 mL) at 0°C . The reaction mixture was allowed to warm to rt. After stirring for 40 min, the mixture was diluted with EtOAc, filtered through Celite, concentrated, and partitioned between brine and CH_2Cl_2 . The separated aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were dried over Na_2SO_4 and concentrated to furnish the crude aldehyde intermediate.

NaBH_4 (228 mg, 6.0 mmol) was added to a solution of the aldehyde in $\text{MeOH}-\text{Et}_2\text{O}$ (1:1, 40 mL) at 0°C . The mixture was stirred for 30 min, carefully quenched with saturated aqueous NH_4Cl , stirred for 20 min at rt and extracted with CH_2Cl_2 (3x). The combined extracts were dried over Na_2SO_4 , concentrated and purified by flash chromatography (40% to 50% EtOAc-hexanes) to afford alcohol X-20 (1.02 g, 93% for two steps).

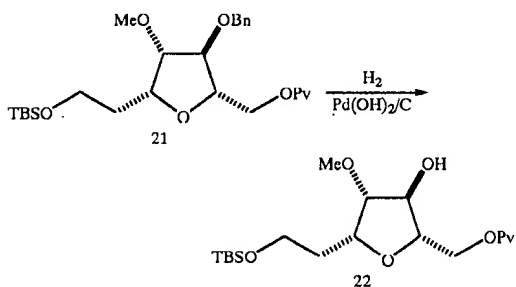


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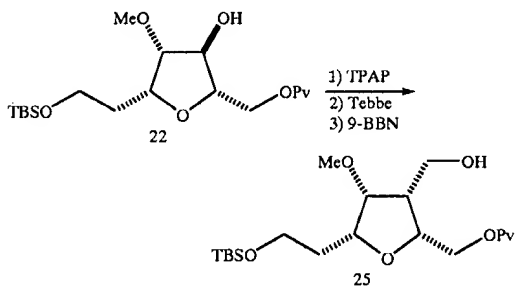
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Silyl ether 21 Imidazole (0.94 g, 13.9 mmol) and TBSCl (0.59 g, 3.89 mmol) were added sequentially to a solution of alcohol X-20 (1.02 g, 2.78 mmol) in DMF (10 mL) at rt. After 14 h, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc (3 \times). The combined organic extracts were washed with H_2O , brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (5% to 15% EtOAc-hexanes) to afford silyl ether 21 (1.3 g, 98%).



Alcohol 22 A mixture of $\text{Pd}(\text{OH})_2$ (20%, 0.8 g), silyl ether 21 (1.3 g, 2.70 mmol) and EtOAc (30 mL) was stirred for 1 h under 1 atm H_2 at rt, filtered through Celite, concentrated and purified by flash chromatography (20% to 40% EtOAc-hexanes) to afford alcohol 22 (0.96 g, 91%).

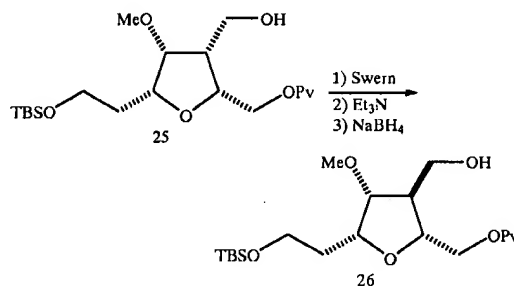


Alcohol 25 4-Methylmorpholine N-oxide (980 mg, 8.4 mmol) and TPAP (131 mg, 3.26 mmol) were added sequentially to a solution of alcohol 22 (1.78 g, 4.6 mmol) in CH_2Cl_2 (45 mL) at rt. A cold bath was necessary to control the exotherm. After 20 min, the reaction mixture was diluted with hexanes, filtered through a short SiO_2 column (15% EtOAc-hexanes) and concentrated to give the crude ketone.

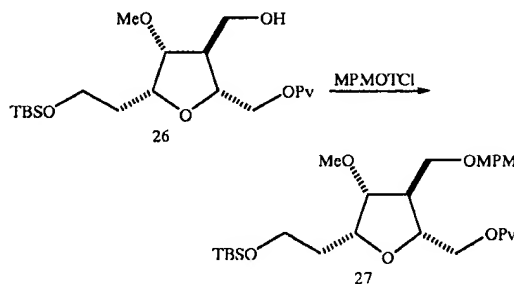
Tebbe reagent (14.9 mL, 9.0 mmol) was added over 10 min to a solution of the crude ketone in THF (60 mL) at 0°C . After 20 min, the reaction mixture was poured into Et_2O (100 mL) that was precooled to -78°C , quenched by slow addition of H_2O (30 mL), warmed to rt., stirred for 30 min and extracted with Et_2O (4 \times). The combined extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (10% EtOAc-hexanes) to afford the desired olefin contaminated by the gem-dimethyl product (1.07 g). This mixture was used directly in the next step.

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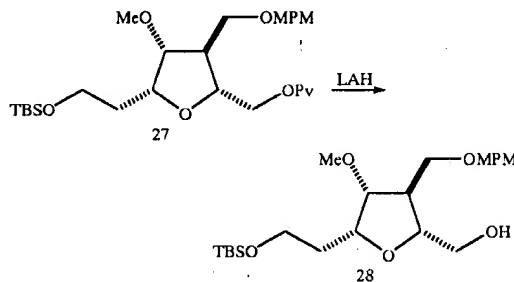
9-BBN (0.5 M in THF, 11.6 mL, 5.8 mmol) was added to a solution of the olefin in THF (15 mL) at 0°C . The reaction mixture was allowed to warm to rt, stirred for 5 h and then recooled to 0°C . H_2O (60 mL), THF (60 mL) and $\text{NaBO}_3\cdot 4\text{H}_2\text{O}$ (5.7 g) were added. After stirring for 5 h at rt, the THF was removed under reduced pressure and the aqueous residue was extracted with EtOAc (4 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (20% to 40% EtOAc-hexanes) to furnish alcohol 25 (605 mg, 18% for three steps).



Alcohol 26 Using the procedure previously described, alcohol 25 (604 mg, 1.49 mmol) was sequentially oxidized, isomerized, and reduced. Purification by flash chromatography (20% to 40% EtOAc-hexanes) afforded alcohol 26 (550 mg, 91% for three steps).



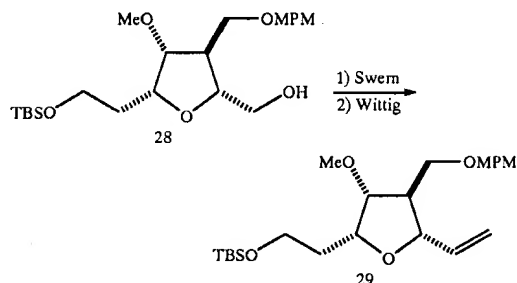
MPM-ether 27 $\text{BF}_3\cdot\text{OEt}_2$ (0.05 M in CH_2Cl_2 , 270 μL , 0.013 mmol) was added to a solution of alcohol 26 (545 mg, 1.35 mmol) and MPM-trichloroimidate (1.14 g, 4.0 mmol) in CH_2Cl_2 (40 mL) at 0°C . After 1 h, the reaction was quenched with saturated aqueous NaHCO_3 , extracted with CH_2Cl_2 , dried over Na_2SO_4 , concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford MPM-ether 27 (580 mg, 82%).



Alcohol 28 LAH (1 M in THF, 1.9 mL, 1.9 mmol) was added to a solution of MPM-ether 27 (580 mg, 1.11 mmol)

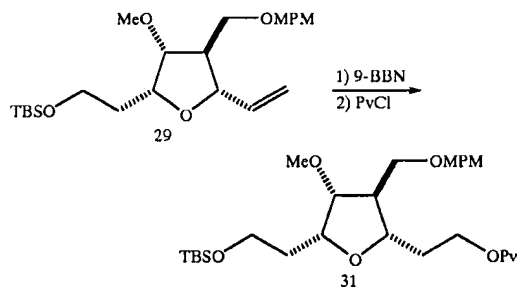
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in Et₂O (100 mL) at 0° C. After 30 min, the reaction was quenched carefully with H₂O (0.5 mL), and 1 N aqueous NaOH (0.5 mL), stirred for 1 h at rt, filtered through Celite, concentrated and purified by flash chromatography (30% to 50% EtOAc-hexanes) to afford alcohol 28 (460 mg, 95%).



Olefin 29 DMSO (441 μ L, 6.23 mmol) was added to a solution of oxalyl chloride (272 μ L, 3.12 mmol) in CH₂Cl₂ (30 mL) at -78° C. After 15 min, a solution of alcohol 28 (458 mg, 1.04 mmol) in CH₂Cl₂ (15 mL) was added to the reaction mixture. After stirring for 1 h at -78° C., Et₃N (1.3 mL, 9.35 mmol) was added. The reaction mixture was warmed to 0° C., stirred for 10 min, diluted with saturated aqueous NH₄Cl and extracted with CH₂Cl₂ (3 \times). The combined organic extracts were dried over Na₂SO₄, concentrated and filtered through a short SiO₂ column (20% to 30% EtOAc-hexanes) to provide the crude aldehyde.

n-BuLi (1.63 M, 1.4 mL, 2.28 mmol) was added dropwise to a solution of CH₃PPh₃Br (815 mg, 2.28 mmol), THF (20 mL) and DMSO (7.5 mL) at 0° C. After 1 h, a solution of the aldehyde in THF (10 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h. Saturated aqueous NH₄Cl was added and the mixture was extracted with EtOAc (4 \times). The combined organic extracts were washed with H₂O, brine, dried over Na₂SO₄, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford olefin 29 (380 mg, 95% yield for 2 steps).

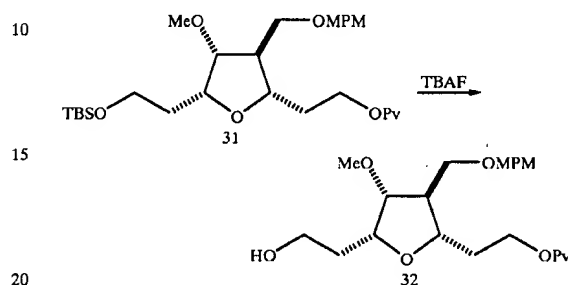


Compound 31 9-BBN (0.5 M in THF, 6 mL, 3 mmol) was added to a solution of olefin 29 (370 mg, 0.85 mmol) in THF (7 mL) at 0° C. The mixture was allowed to warm to rt and stirred for 1 h. After recooling to 0° C., H₂O (30 mL), THF (20 mL), and NaBO₃·4 H₂O (2.8 g) were added. After stirring for 3 h at rt, the THF was removed under reduced pressure. The aqueous residue was extracted with EtOAc (4 \times), dried over Na₂SO₄, concentrated and purified by flash chromatography (25% to 50% EtOAc-hexanes) to afford alcohol 30 which was used directly in the next step.

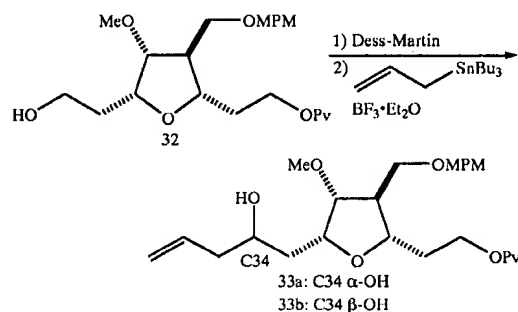
Pivaloyl chloride (157 μ L, 1.27 mmol) was added to a solution of alcohol 30 in CH₂Cl₂-pyridine (1:1 mixture, 10 mL) at rt. After 18 h, additional pivaloyl chloride (100 μ L,

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0.81 mmol) was added. After 1 h, the reaction mixture was cooled to 0° C., quenched with MeOH (0.5 mL), concentrated, diluted with brine and extracted with CH₂Cl₂ (4 \times). The combined organic extracts were dried over Na₂SO₄, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford compound 31 (410 mg, 90% for two steps).



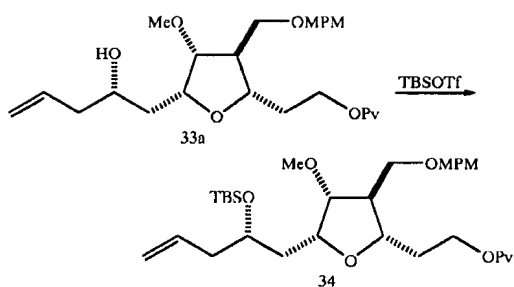
Alcohol 32 TBAF (1 M in THF, 1.14 mL, 1.14 mmol) was added to a solution of 31 (410 mg, 0.761 mmol) in THF (5 mL) at rt. After 1.5 h, the reaction mixture was concentrated and purified by flash chromatography (40% EtOAc-hexanes to 100% EtOAc) to afford alcohol 32 (320 mg, 100%).



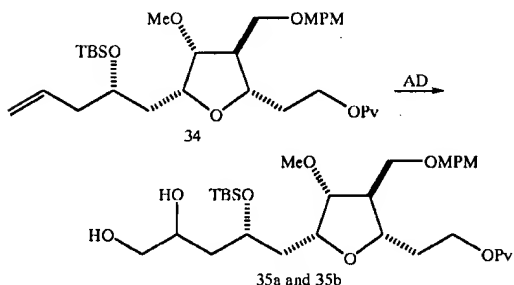
Alcohols 33a and 33b Dess-Martin periodinane (925 mg, 2.18 mmol) was added to a solution of alcohol 32 (309 mg, 0.727 mmol) in CH₂Cl₂ (19 mL) at rt. After 1 h, the reaction was diluted with Et₂O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous NaHCO₃-Na₂S₂O₃ and brine, dried over Na₂SO₄, concentrated and purified by flash chromatography (20% to 30% EtOAc-hexanes) to afford the desired aldehyde, which was taken immediately through the next step.

BF₃·OEt₂ (135 μ L, 1.1 mmol) was added to a solution of the crude aldehyde, tri-n-butylallyltin (337 μ L, 1.08 mmol) and CH₂Cl₂ (16 mL) at -78° C. After 1 h, the reaction was quenched with saturated aqueous NaHCO₃ and extracted with CH₂Cl₂ (3 \times). The combined organic extracts were dried over Na₂SO₄, concentrated and purified by MPLC (25% to 30% EtOAc-hexanes) to afford the major, more polar alcohol 33a (165 mg, 49% for two steps) and the minor less polar product 33b (90 mg, 27% for two steps).

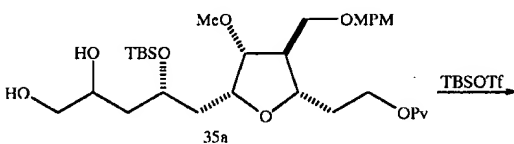
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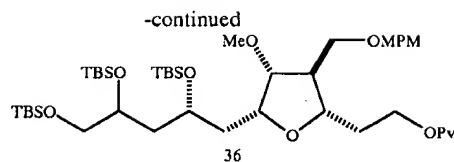
Compound 34 TBSOTf (163 μ L, 0.710 mmol) was added to a solution of alcohol 33a (165 mg, 0.355 mmol), Et₃N (247 μ L, 1.78 mmol) and CH₂Cl₂ (5 mL) at 0° C. After 25 min, the reaction was quenched with saturated aqueous NaHCO₃, extracted with CH₂Cl₂ (3 \times), dried over Na₂SO₄, concentrated and purified by flash chromatography (15% to 20% EtOAc-hexanes) to afford compound 34 (200 mg, 98%).



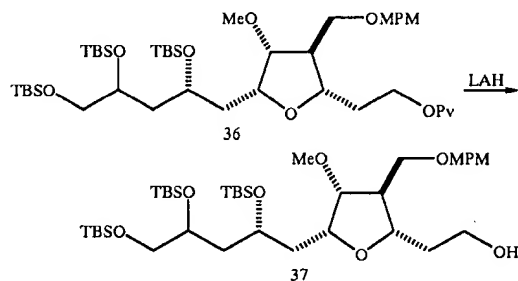
Diols 35a and 35b OsO₄ (0.1 M solution in toluene, 32 μ L, 3.2 μ mol) was added to a solution of K₂CO₃ (168 mg, 1.22 mmol), K₃Fe(CN)₆ (400 mg, 1.22 mmol), (DHQ)₂PYR (11 mg, 12 μ mol), H₂O (3.2 mL) and t-BuOH (2.2 mL) at 0° C. Then a solution of olefin 34 (200 mg, 0.345 mmol) in t-BuOH (1 mL) was added to the reaction mixture. After 5 h at 0° C., Na₂S₂O₅·5 H₂O (200 mg) was added. The reaction mixture was warmed to rt, stirred for 30 min and extracted with CH₂Cl₂ (5 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by preparative TLC (70% EtOAc-hexanes) to afford the major, less polar diol 35a (118 mg, 56%), and minor, more polar diastereomeric product 35b (74 mg, 35%). The individual diastereomers were each carried forward separately.



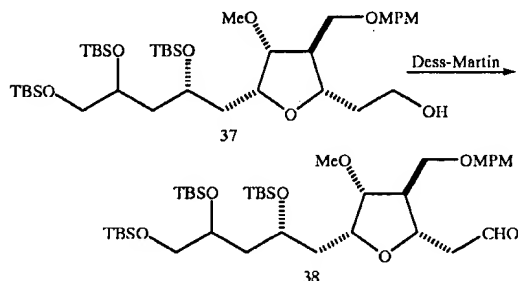
100



Compound 36 TBSOTf (177 μ L, 0.77 mmol) was added to a solution of diol 35a (118 mg, 0.192 mmol), Et₃N (267 μ L, 1.92 mmol) and CH₂Cl₂ (5 mL) at 0° C. After 25 min, the reaction was quenched with saturated aqueous NaHCO₃, extracted with CH₂Cl₂ (3 \times), dried over Na₂SO₄, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford compound 36 (161 mg, 100%).



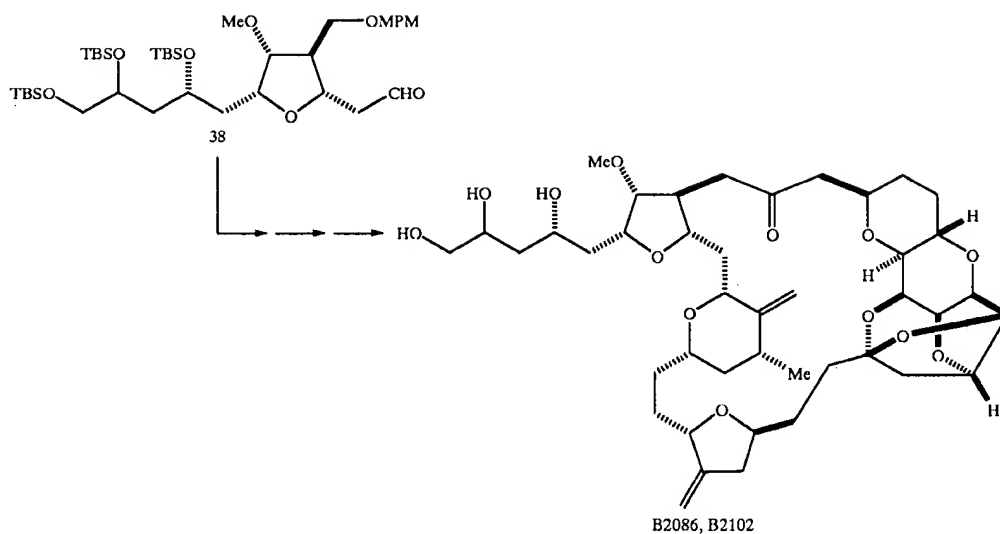
Alcohol 37 Using the procedure described previously for the preparation of alcohol 28, compound 36 (161 mg, 0.192 mmol) afforded alcohol 37 (135 mg, 93%) after purification by flash chromatography (20% to 40% EtOAc-hexanes).



Aldehyde 38 Dess-Martin periodinane (227 mg, 0.535 mmol) was added to a solution of alcohol 37 (135 mg, 0.178 mmol) in CH₂Cl₂ (5 mL) at rt. After 1 h, the reaction mixture was diluted with Et₂O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous NaHCO₃—Na₂S₂O₃ and brine, dried over Na₂SO₄, concentrated and purified by flash chromatography (10% to 20% EtOAc-hexanes) to afford aldehyde 38 (127 mg, 95%).

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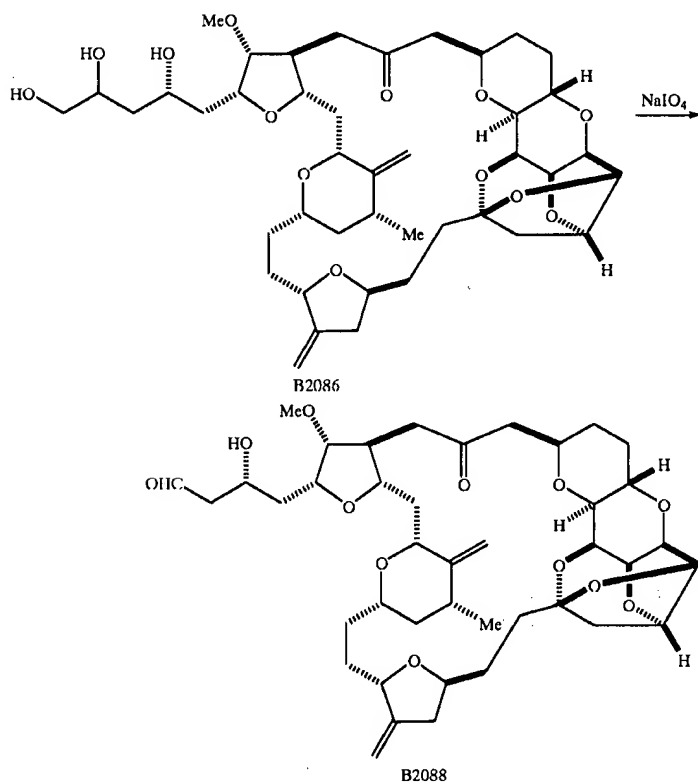
102



B2086, B2102 Each of the diastereomers obtained above were separately carried to final product in a manner similar to that described in scheme 6 for B1794. Diastereomer 35a afforded B2086. Diastereomer 35b afforded B2102.

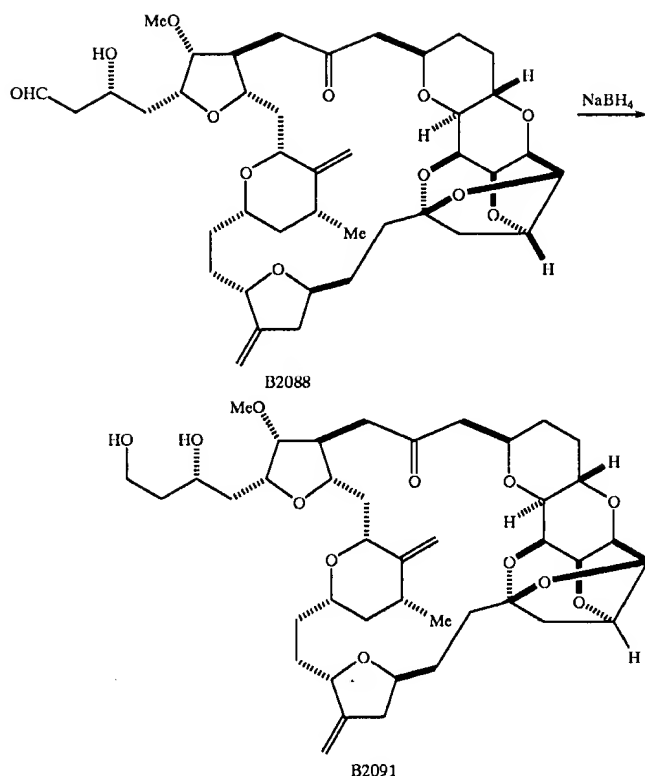
25

B2088 NaIO₄ was added to a solution of B2086 (1 mg, 1.29 μmol) in MeOH—H₂O (4:1, 1 mL) at rt. After 30 min, the reaction mixture was diluted with H₂O, extracted with CH₂Cl₂ (6×), dried over Na₂SO₄, and concentrated to afford B2088 (1.2 mg).



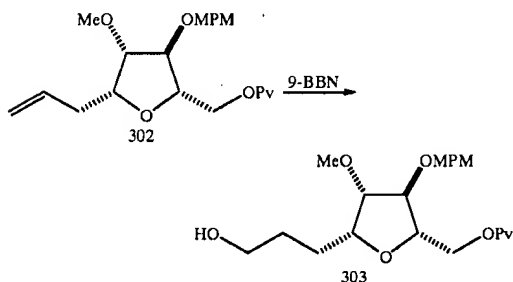
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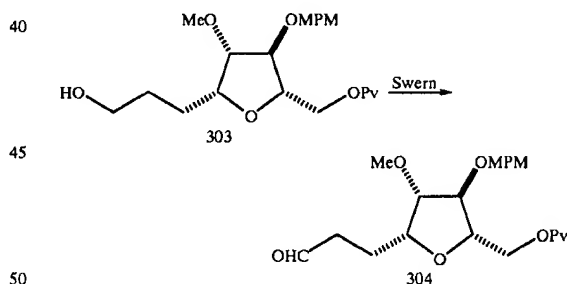
B2091 NaBH_4 (0.013 M in EtOH, 20 μL , 0.27 μmol) was added to a solution of B2088 (1 mg, 1.29 μmol) in MeOH— CH_2Cl_2 (4:1, 0.5 mL) at -78°C . Additional NaBH_4 was periodically added with close monitoring of the reaction by TLC (total of 220 μL of the NaBH_4 solution was required). The reaction mixture was quenched at 0°C with saturated aqueous NH_4Cl , stirred for 20 min at rt and extracted with CH_2Cl_2 (6 \times). The combined extracts were dried over Na_2SO_4 , concentrated and purified by preparative TLC (7% MeOH—EtOAc) to furnish B2091 (0.40 mg, 50%).

Synthesis of B1933:



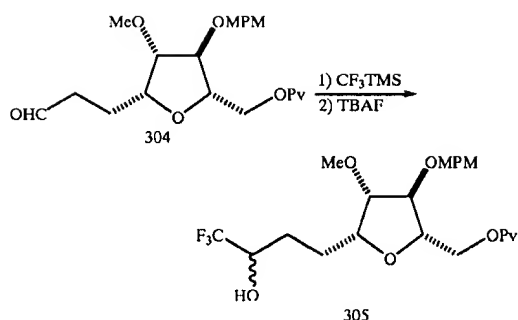
Alcohol 303 9-BBN (0.5 M in THF, 23 mL, 0.012 mol) was added dropwise over 30 min to a solution of alkene 302 (1.51 g, 0.00386 mol) in THF (40 mL) at 0°C . After stirring at rt for 80 min, the mixture was cooled to 0°C and H_2O (80 mL) was cautiously added followed by $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ (4.2 g, 0.027 mol). The mixture was stirred vigorously at rt for 2.3 h, then extracted with EtOAc (3 \times). The combined organic extracts were washed with brine, dried over

Na_2SO_4 , concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol 303 (1.37 g, 87%).

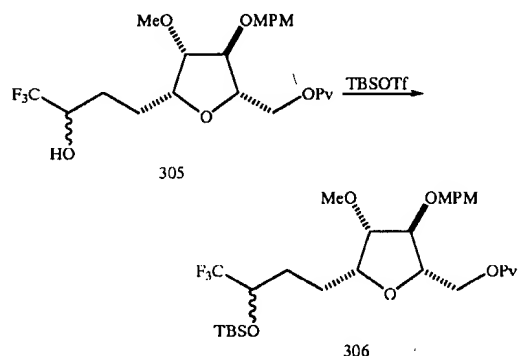


Aldehyde 304 Oxalyl chloride (88 μL , 1.00 mmol) was added dropwise to a solution of DMSO (142 μL , 2.00 mmol) in CH_2Cl_2 (20 mL) at -78°C . After 30 min, a solution of alcohol 303 (137 mg, 0.335 mmol) in CH_2Cl_2 (5 mL) was added and stirred at -78°C for 1 h. Et_3N (420 μL , 3.01 mmol) was added and after 10 min the reaction was stirred for 10 min at 0°C at which point saturated aqueous NH_4Cl was added and the resulting mixture was extracted with CH_2Cl_2 (3 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (50% EtOAc-hexanes) to provide intermediate aldehyde 304 (0.114 g, 84%) which was immediately used in the next step.

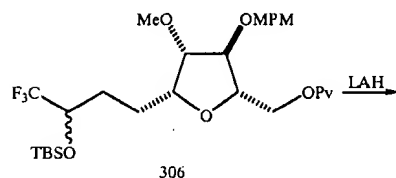
105



Alcohol 305 TBAF (1 M in THF, 5 μL , 0.005 mmol) was added to a solution of aldehyde 304 (0.114 g, 0.27 mmol) in CF_3TMS (0.5 M in THF, 1.1 mL, 0.54 mmol) at 0°C . After 20 min, a second portion of TBAF (1 M in THF, 100 μL , 0.1 mmol) was added and the mixture was stirred for 10 min at which point excess TBAF (1 M in THF, 270 μL , 0.27 mmol) was added dropwise to cleave the intermediate silyl ether. After 30 min, the mixture was diluted with H_2O and extracted with EtOAc (3 \times). The organic extracts were washed with H_2O , brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol 305 (123 mg, 95%) as an inseparable 1:1 mixture of isomers.

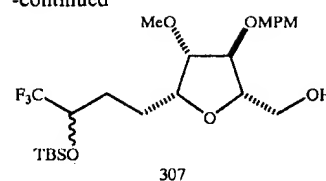


Silyl ether 306 TBSOTf (265 μL , 1.16 mmol) was added to a solution of alcohol 305 (123 mg, 0.257 mmol) and Et_3N (430 μL , 3.08 mmol) in CH_2Cl_2 (8 mL) at 0°C . After stirring at rt for 20 h, saturated aqueous NaHCO_3 was added, and the mixture was extracted with CH_2Cl_2 (3 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide silyl ether 306 (148 mg, 97%).

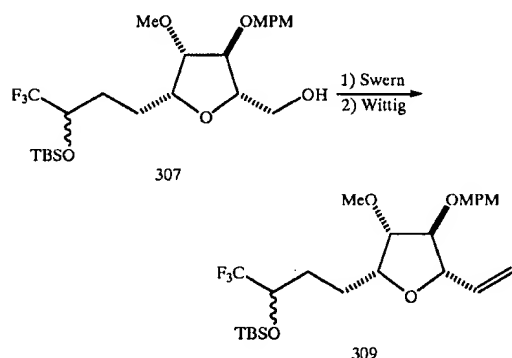


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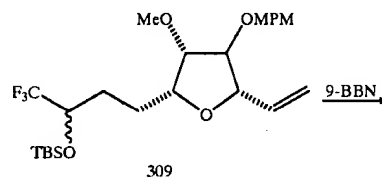


Alcohol 307 LAH (1 M in THF, 220 μL , 0.22 mmol) was added dropwise to a solution of silyl ether 306 (131 mg, 0.22 mmol) in Et_2O (5 mL) at 0°C . After 30 min, H_2O and 1 M NaOH were cautiously added. The mixture was stirred at rt 30 min, filtered through glass wool, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol 307 (112 mg, quant.).

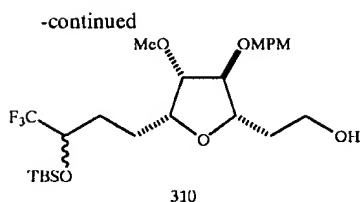


Alkene 309 Oxalyl chloride (58 μL , 0.66 mmol) was added dropwise to a solution of DMSO (94 μL , 1.3 mmol) in CH_2Cl_2 (10 mL) at -78°C . After 30 min, a solution of alcohol 307 (112 mg, 0.22 mmol) in CH_2Cl_2 (3 mL) was added. After 1 h, Et_3N (276 μL , 1.98 mmol) was added, and after 10 min at -78°C , the reaction was stirred at 0°C for 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (50% EtOAc-hexanes) to provide aldehyde 308 (101 mg, 91%) which was immediately used in the next step.

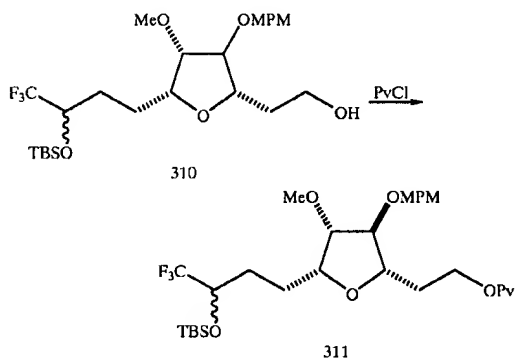
$n\text{BuLi}$ (1.63 M in THF, 200 μL , 0.33 mmol) was added dropwise to a solution of $\text{CH}_3\text{PPh}_3\text{Br}$ (118 mg, 0.33 mmol) in THF (3 mL) and DMSO (1.2 mL) at 0°C . After 70 min, a solution of aldehyde 308 (101 mg, 0.20 mmol) in THF (3 mL) was added and after 10 min at 0°C , the reaction was stirred at rt for 1 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (3 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alkene 309 (90.9 mg, 90%).



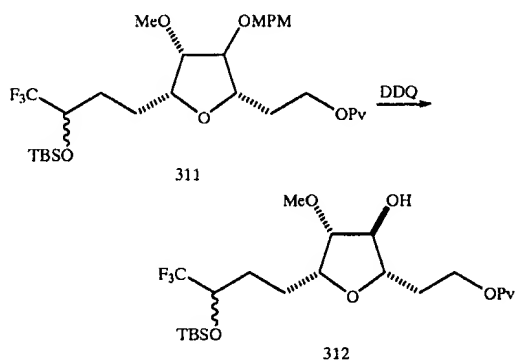
107



Alcohol 310 9-BBN (0.5 M in THF, 17 mL, 8.45 mmol) was added dropwise to a solution of alkene 309 (1.06 g, 2.11 mmol) in THF (30 mL) at 0° C. After stirring for 2.5 h at rt, the reaction was cooled to 0° C. and H₂O (60 mL) followed by NaBO₃·4 H₂O (3.25 g, 21.1 mmol) were cautiously added. The mixture was stirred vigorously at rt for 2 h, then diluted with H₂O and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol 310 (0.920 g, 84%).



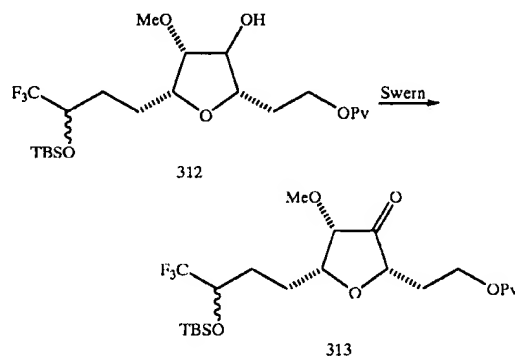
Pivalate 311 A mixture of alcohol 310 (65.8 mg, 0.0126 mmol), pyridine (61 μL, 0.76 mmol) and PivCl (23 μL, 0.189 mmol) in CH₂Cl₂ (3 mL) was stirred at rt for 5 h. A second reaction utilizing alcohol 310 (0.92 g, 1.76 mmol) was run under similar conditions and both reactions were combined during the work-up: saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide pivalate 311 (1.08 g, quant).



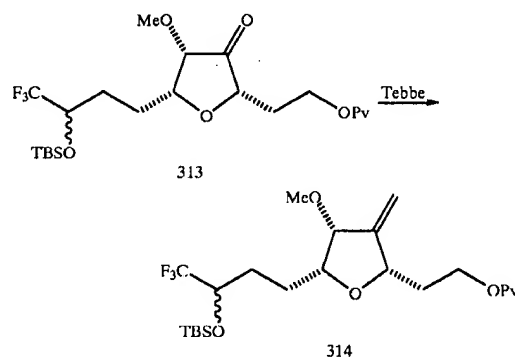
Alcohol 312 A mixture of ether 311 (0.811 g, 1.33 mmol), DDQ (6.1 g, 27 mmol) and 10:1 tBuOH: pH 7 phosphate

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buffer (42 mL) in CH₂Cl₂ (84 mL) was stirred vigorously in the dark at rt for 1.5 h, at which point additional DDQ (1.0 g, 4.4 mmol) was added. After 1 h, saturated aqueous NaHCO₃ was added and the mixture was extracted with CH₂Cl₂ (4×). The combined organic extracts were washed successively with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alcohol 312 (0.56 g, 87%) as well as recovered starting material 311 (97 mg, 12%).

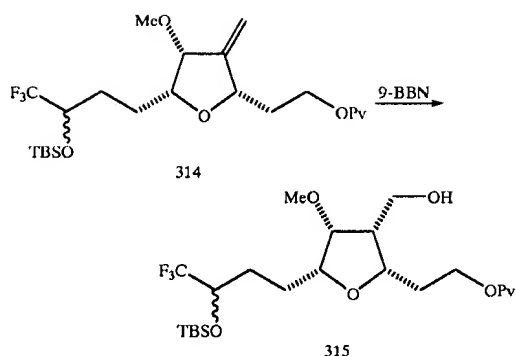


Ketone 313 Oxalyl chloride (21 μL, 0.12 mmol) was added dropwise to a solution of DMSO (34 μL, 0.48 mmol) in CH₂Cl₂ (3 mL) at -78° C. After 1 h, a solution of alcohol 312 (39.4 mg, 0.081 mmol) in CH₂Cl₂ (1.5 mL) was added and the mixture was stirred for 1.5 h. Et₃N (100 μL, 0.73 mmol) was added, and after 10 min the mixture was warmed to 0° C. Saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide ketone 313 (36.6 mg, 93%) which was used immediately in the next step.

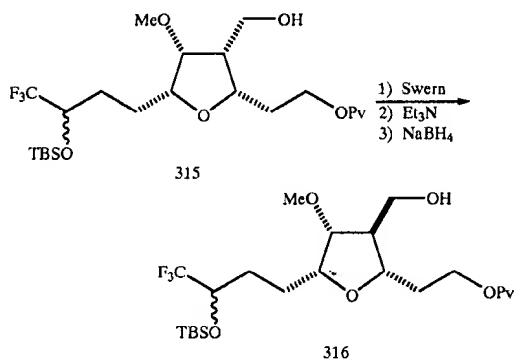


Alkene 314 Tebbe reagent (~0.65 M in toluene, 720 μL, 0.47 mmol) was added dropwise to a solution of ketone 313 (151 mg, 0.31 mmol) in THF (5 mL) at 0° C. After 15 min, H₂O was cautiously added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (10% EtOAc-hexanes) to provide alkene 314 (139 mg, 93%).

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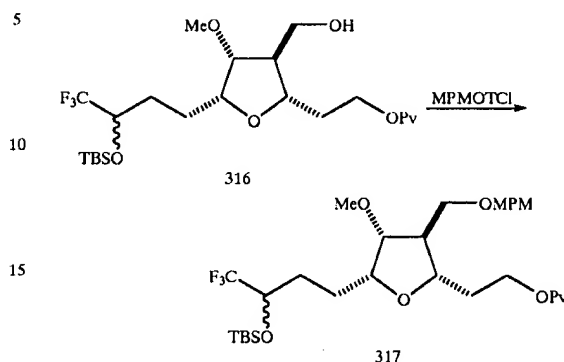
Alcohol 315 9-BBN (0.5 M in THF, 6.0 mL, 2.9 mmol) was added dropwise to a solution of alkene 314 (468 mg, 0.97 mmol) in THF (10 mL) at 0° C. The mixture was stirred at rt for 2 h at which point additional 9-BBN (0.5 M in THF, 500 μ L, 0.25 mmol) was added. After 2.5 h, the mixture was cooled to 0° C. and H₂O (10 mL) followed by NaBO₃·4 H₂O (1.5 g, 9.7 mmol) were cautiously added. The mixture was stirred vigorously at rt for 5 h, diluted with H₂O and extracted with EtOAc (3 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (gradient 20% to 30% EtOAc-hexanes) to provide alcohol 315 (0.47 g, 97%).



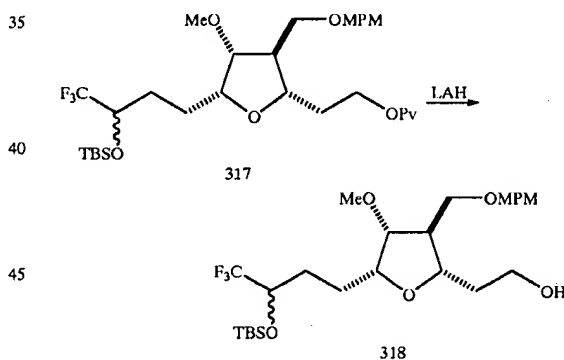
Alcohol 316 Oxalyl chloride (246 μ L, 2.82 mmol) was added dropwise to a solution of DMSO (400 μ L, 5.64 mmol) in CH₂Cl₂ (40 mL) at -78° C. After 1 h, a solution of alcohol 315 (0.47 g, 0.94 mmol) in CH₂Cl₂ (10 mL) was added and the mixture was stirred for 1 h. Et₃N (1.2 mL, 8.5 mmol) was added, and after 10 min the mixture was warmed to 0° C. and stirred for 10 min. Saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude aldehyde was stirred in CH₂Cl₂ (20 mL) and Et₃N (2 mL) at rt overnight. Saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by flash chromatography (30% EtOAc-hexanes) provided the epimerized aldehyde which was immediately dissolved in 1:1 Et₂O:EtOH (10 mL) and cooled to 0° C. NaBH₄ (35 mg, 0.94 mmol) was added and after 10 min the reaction was quenched with saturated aqueous NH₄Cl. The mixture was extracted with EtOAc (3 \times) and the combined organic extracts were washed with

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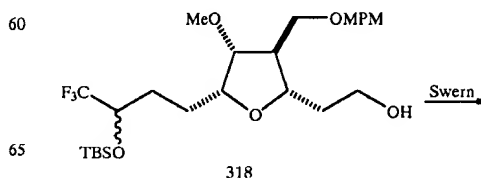
brine, dried over Na₂SO₄, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol 316 (0.410 g, 87% yield for 3 steps).



Ether 317 Alcohol 316 (60.7 mg, 0.12 mmol) and MPMOTCl (0.10 g, 0.36 mmol) were combined, azeotroped from toluene (3 \times) and dried under high vacuum overnight. CH₂Cl₂ (3 mL) was added and the mixture was cooled to 0° C. BF₃·OEt₂ (approx. 1 μ L, 0.01 mmol) was added and after stirring for 10 min the reaction was quenched with saturated aqueous NH₄Cl. The mixture was extracted with CH₂Cl₂ (3 \times) and the combined extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide ether 317 (55.4 mg, 74%).

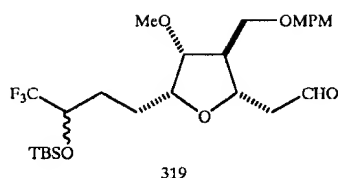


Alcohol 318 LAH (1 M in THF, 104 μ L, 0.104 mmol) was added dropwise to a solution of ether 317 (54 mg, 0.087 mmol) in Et₂O (5 mL) at 0° C. After 30 min, H₂O and 1 M NaOH were cautiously added. The mixture was stirred at rt for 10 min, filtered through glass wool, concentrated and purified by column chromatography (30%–50% EtOAc-hexanes) to provide alcohol 318 (45.5 mg, 98%).

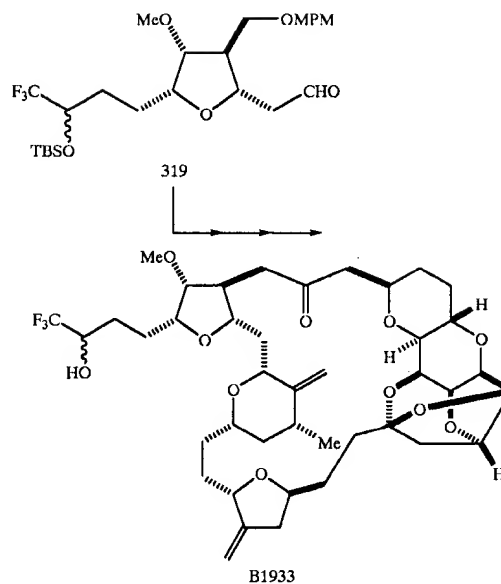


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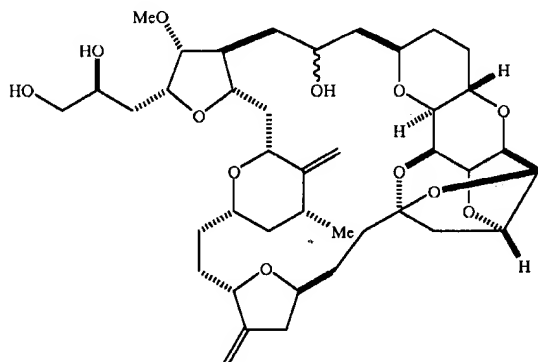


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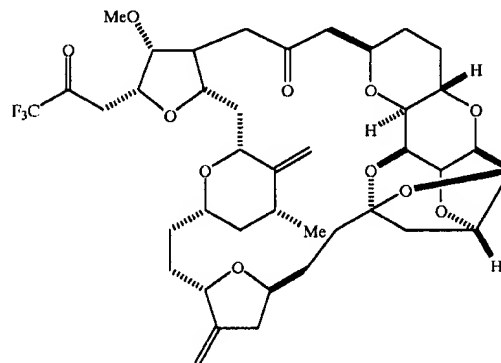
Aldehyde 319 Oxalyl chloride (11 μ L, 0.13 mmol) was added dropwise to a solution of DMSO (18 μ L, 0.25 mmol) in CH_2Cl_2 (2 mL) at -78°C . After 1.8 h, a solution of alcohol 318 (22.6 mg, 0.042 mmol) in CH_2Cl_2 (1 mL) was added and the mixture was stirred for 1 h. Et_3N (53 μ L, 0.38 mmol) was added and after 10 min, the reaction was warmed to 0°C and stirred 10 min. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (20% EtOAc-hexanes) to provide aldehyde 319 (21.7 mg, 97%).

B1933 In a manner similar to that described in Scheme 6 for the synthesis of B1794, intermediate 319 was converted to B1933. HRMS (FAB): calcd for $\text{C}_{41}\text{H}_{57}\text{F}_3\text{O}_{11}+\text{H}$ 783.3931. Found: 783.3940.



B1897

- 1) NaIO_4
- 2) CF_3TMS
- 3) TBAF
- 4) Dess-Martin



B1942

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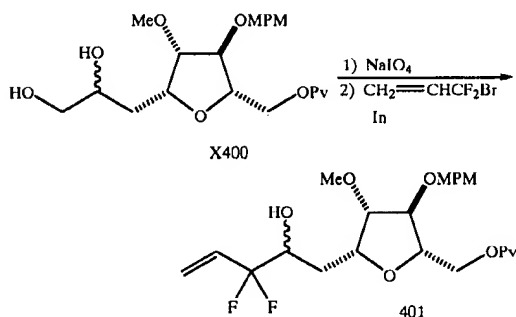
B1942 A mixture of B1897 (2 mg, 2.73 μ mol), NaIO_4 (35 mg, 0.16 mmol), MeOH (0.8 mL) and H_2O (0.2 mL) was stirred at rt for 30 min. The reaction mixture was then diluted with H_2O (3 mL) and extracted with CH_2Cl_2 (6 \times) and EtOAc (2 \times). The combined organic phases were dried over Na_2SO_4 and purified by column chromatography (5% MeOH— CH_2Cl_2) to give the desired aldehyde.

This material was dissolved in THF (0.1 mL), cooled to at 0° C. and treated with 0.5 M CF_3TMS in THF (30 μ L, 15 mmol) followed by 0.05 M TBAF in THF (5 mL, 0.025 mmol). After stirring for 30 min, the reaction mixture was diluted with saturated aqueous NaHCO_3 (2 mL) and H_2O (1 mL), extracted with EtOAc (6 \times), dried over Na_2SO_4 , filtered and concentrated to give the crude bis-TMS ether.

This material was dissolved in THF (0.5 mL) and treated with 1 M TBAF in THF containing 0.5 M imidazole hydrochloride (8 μ L, 8 μ mol) at rt for 30 min. The reaction mixture was eluted through a SiO_2 column (50% EtOAc-hexanes to EtOAc) to afford the diol intermediate.

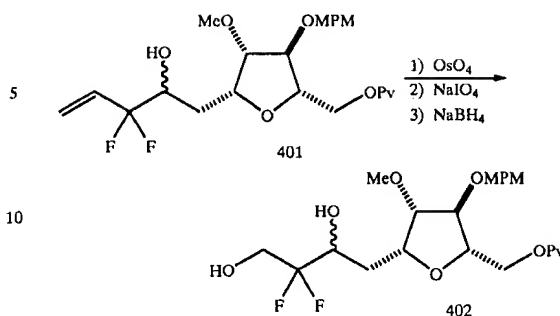
A mixture of this product and Dess-Martin periodinane (10 mg, 24 mmol) in CH_2Cl_2 (0.5 mL) was stirred at rt for 1 h, diluted with Et₂O (5 mL) and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to finish B1942 (1.5 mg, 72% for 5 steps). HRMS (FAB): calcd for $\text{C}_{40}\text{H}_{55}\text{F}_3\text{O}_{11}+\text{H}$ 767.3516. Found: 767.3542

Synthesis of B2070/B2073:

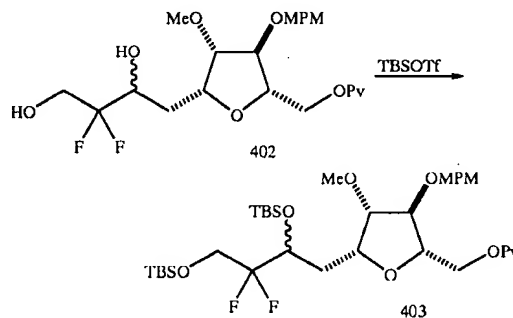


Alcohol 401 A mixture of NaIO_4 (375 mg, 1.74 mmol), X400 (674 mg, 1.58 mmol), MeOH (16 mL) and H_2O (4 mL) was stirred at rt for 1 h. After dilution with H_2O , the mixture was extracted with CH_2Cl_2 (4 \times) and the combined organic extracts were dried over Na_2SO_4 , concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide the intermediate aldehyde (570 mg), which was immediately dissolved in DMF (15 mL). Indium (275 mg, 2.4 mmol) and 3-bromo-3,3-difluoropropene (240 μ L, 2.4 mmol) were added and after stirring at rt for 17 h, H_2O and 0.1 M HCl were added. The mixture was extracted with EtOAc (3 \times) and the combined organic extracts were washed successively with H_2O and brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol 401 as a 1:1 mixture of C34 isomers (605 mg, 81% for 2 steps).

114

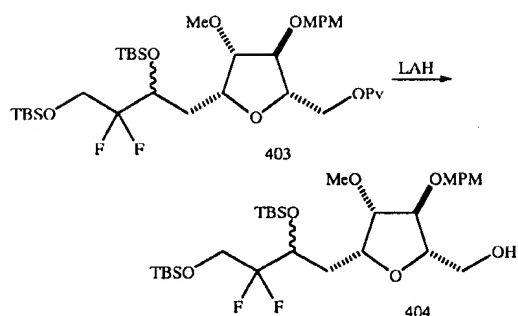


Diol 402 A mixture of OsO_4 (1 xstal), alcohol 401 (605 mg, 1.28 mmol), 4-methyl-morpholine N-oxide (0.45 g, 3.84 mmol), acetone (30 mL) and H_2O (6 mL) was stirred at rt for 29 h. Additional OsO_4 (3 xtals) and 4-methylmorpholine N-oxide (0.1 g, 0.8 mmol) were added and after 2 days saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ was added. The mixture was extracted with CH_2Cl_2 (6 \times) and the combined organic extracts were dried over Na_2SO_4 and concentrated. The crude intermediate triol was immediately dissolved in 4:1::MeOH: H_2O (25 mL) and NaIO_4 (0.41 g, 1.9 mmol) was added. After stirring vigorously at rt for 2 h, the mixture was diluted with H_2O , extracted with CH_2Cl_2 (3 \times) and the combined organic extracts were dried over Na_2SO_4 and concentrated to provide the intermediate aldehyde which was immediately dissolved in 1:1 EtOH—Et₂O (30 mL) and cooled to 0° C. NaBH_4 (48 mg, 1.3 mmol) was added and after 20 min the reaction was quenched with H_2O and extracted with CH_2Cl_2 (4 \times). The combined organic extracts were dried over Na_2SO_4 , concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide diol 402 (485 mg, 80% for 3 steps).

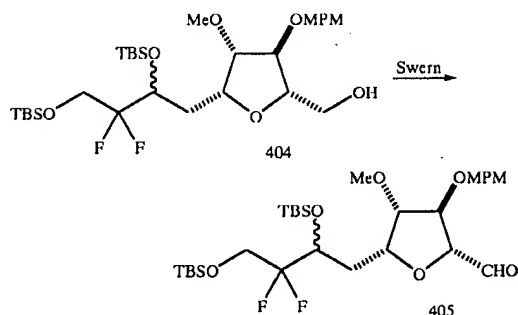


Silyl ether 403 TBSOTf (2.3 mL, 10 mmol) was added dropwise to a mixture of diol 402 (485 mg, 1.0 mmol), Et₃N (2.8 mL, 20 mmol) and CH_2Cl_2 (30 mL) at 0° C. After stirring for 1 h at rt, saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2 (3 \times). The combined organic extracts were washed with brine, dried over Na_2SO_4 , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide silyl ether 403 (668 mg, 95%).

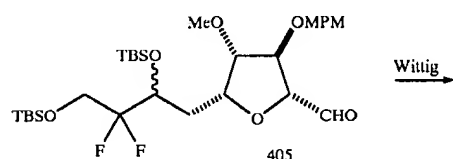
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Alcohol 404 LAH (1 M in THF, 2.8 mL, 2.8 mmol) was added dropwise to a solution of silyl ether 403 (668 mg, 0.948 mmol) in Et₂O (60 mL) at 0° C. After 15 min, H₂O and 1 M NaOH were cautiously added. The mixture was stirred at rt for 20 min, filtered through glass wool, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol 404 (500 mg, 85%).

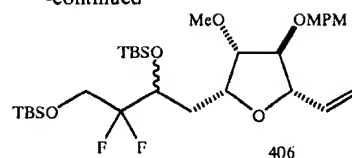


Aldehyde 405 Oxalyl chloride (210 μ L, 2.42 mmol) was added dropwise to a solution of DMSO (345 μ L, 4.84 mmol) in CH₂Cl₂ (30 mL) at -78° C. After 1 h, a solution of alcohol 404 (500 mg, 0.806 mmol) in CH₂Cl₂ (10 mL) was added. After 40 min, Et₃N (1.0 mL, 7.2 mmol) was added. After stirring at -78° C. for 10 min, the reaction mixture was warmed to 0° C. and stirred for an additional 10 min. Saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3 \times). The combined organic extracts were washed successively with H₂O, brine, dried over Na₂SO₄ and concentrated. Purification by flash chromatography (30% EtOAc-hexanes) provided aldehyde 405 (486 mg, 98%) which was immediately used in the next step.

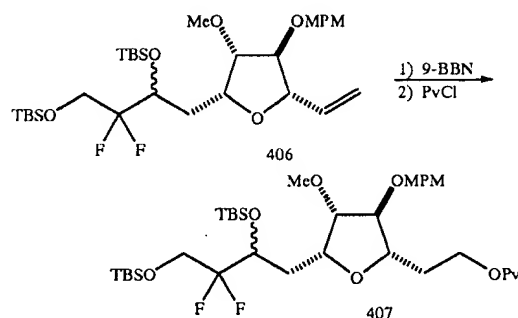


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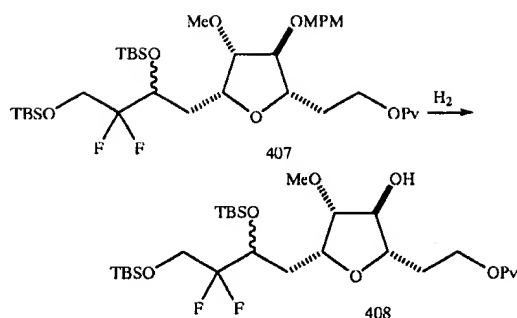


Alkene 406 nBuLi (1.63 M, 860 μ L, 1.4 mmol) was added dropwise to a solution of CH₃PPh₃Br (500 mg, 1.4 mmol) in THF (15 mL) and DMSO (6 mL) at 0° C. After 1 h, a solution of aldehyde 405 (486 mg) in THF (15 mL) was added. The reaction mixture was warmed to rt and stirred for 30 min. Saturated aqueous NH₄Cl was added, the mixture was extracted with EtOAc (3 \times) and the combined extracts were washed successively with H₂O and brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alkene 406 (450 mg, 93%).

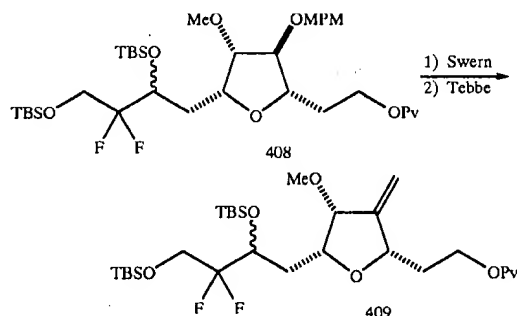


Ester 407 9-BBN (0.5 M in THF, 9.0 mL, 4.5 mmol) was added dropwise to a solution of alkene 406 (0.460 g, 0.746 mmol) in THF (10 mL) at 0° C. After warming to rt, the mixture was stirred for 3 h and two additional portions of 9-BBN (0.5 M in THF, 3.0 mL, 1.5 mmol) were added at 30 min intervals. The reaction mixture was recooled to 0° C., whereupon THF (10 mL), H₂O (10 mL) and NaBO₃·4 H₂O (1.72 g, 11.2 mmol) were cautiously added. The mixture was stirred vigorously at rt for 1.5 h, and additional NaBO₃·4 H₂O (1.0 g, 6.5 mmol) was added. After 2 h the mixture was diluted with H₂O and extracted with EtOAc (3 \times). The combined extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide the intermediate alcohol (509 mg) which was immediately dissolved in CH₂Cl₂ (10 mL) and treated with pyridine (600 μ L, 7.5 mmol) and PvCl (275 μ L, 2.2 mmol). After 6 h, saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide ester 407 (423 mg, 79% for 2 steps).

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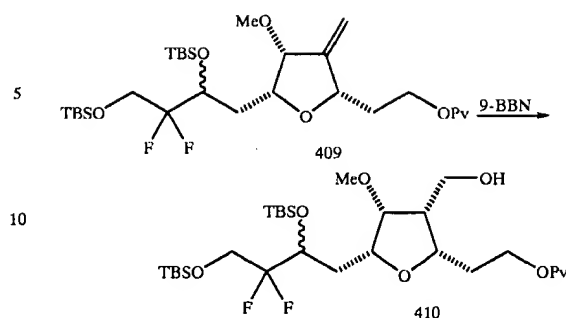


Alcohol 408 A mixture of ester 407 (11 mg, 0.015 mmol) and Pd(OH)₂/C (10 mg) in EtOAc (500 μ L) was stirred vigorously under a H₂ atmosphere at rt for 6 h. The mixture was filtered through Celite, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol 408 (9.4 mg, quant).

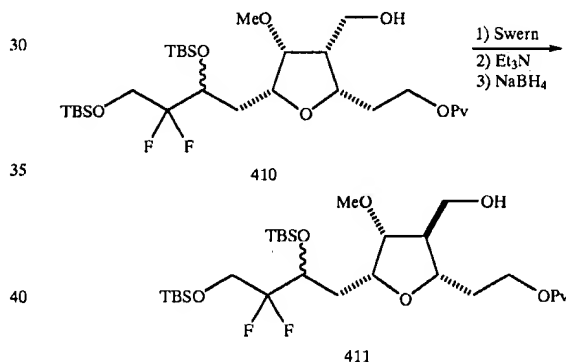


Alkene 409 Oxalyl chloride (7 μ L, 0.075 mmol) was added dropwise to a solution of DMSO (11 μ L, 0.15 mmol) in CH₂Cl₂ (2 mL) at -78° C. under N₂. After 40 min, a solution of alcohol 408 (15.2 mg, 0.025 mmol) in CH₂Cl₂ (1 mL) was added and the reaction was stirred at -78° C. for 1 h. Et₃N (31 μ L, 0.22 mmol) was added, and after stirring for 10 min the mixture was warmed to 0° C. After 10 min, the reaction mixture was quenched with saturated aqueous NH₄Cl and extracted with CH₂Cl₂ (3 \times). The combined extracts were washed successively with H₂O and brine, dried over Na₂SO₄ and concentrated. After flash chromatography (30% EtOAc-hexanes), the intermediate ketone (13 mg) was immediately dissolved in TMF (500 μ L) and treated with Tebbe reagent (~0.65 M in toluene, 62 μ L, 0.040 mmol) at 0° C. After 1.5 h additional Tebbe reagent (~0.65 M in toluene, 62 μ L, 0.040 mmol) was added and after 10 min H₂O and then brine were cautiously added. The mixture was extracted with EtOAc (3 \times) and the combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (10% EtOAc-hexanes) to provide alkene 409 (11.9 mg, 80% for 2 steps).

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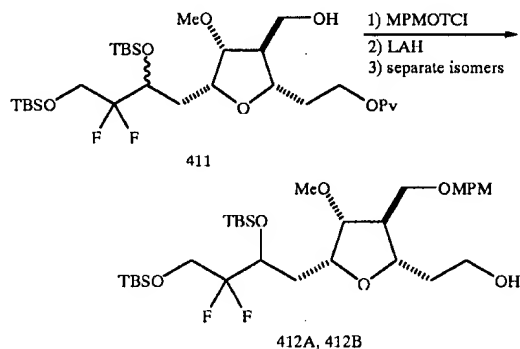
Alcohol 410 9-BBN (0.5 M in THF, 1.5 mL, 0.72 mmol) was added dropwise to a solution of alkene 409 (0.144 g, 0.242 mmol) in THF (2 mL) at 0° C. After warming to rt, the mixture was stirred for 3 h. The reaction mixture was recooled to 0° C., whereupon THF (2 mL), H₂O (2 mL) and NaBO₃·4 H₂O (0.38 g, 2.4 mmol) were cautiously added. The mixture was stirred vigorously at rt for 4 h, diluted with H₂O and extracted with EtOAc (3 \times). The combined extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alcohol 410 (0.140 g, 94%).



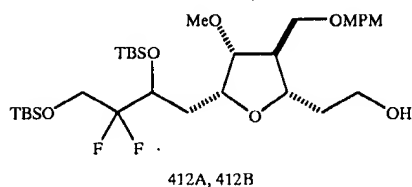
Alcohol 411 Oxalyl chloride (26 μ L, 0.30 mmol) was added dropwise to a solution of DMSO (43 μ L, 0.60 mmol) in CH₂Cl₂ (4 mL) at -78° C. After 1 h, a solution of alcohol 410 (57 mg, 0.093 mmol) in CH₂Cl₂ (2 mL) was added. After 45 min, Et₃N (125 μ L, 0.90 mmol) was added. After stirring at -78° C. for 10 min, the reaction mixture was warmed to 0° C. and stirred for an additional 10 min. Saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was dissolved in CH₂Cl₂ (4 mL), treated with Et₃N (400 μ L) and stirred at rt for 15 h. Saturated aqueous NH₄Cl was added and the mixture was extracted with CH₂Cl₂ (3 \times). The combined organic extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide the intermediate aldehyde (48 mg), which was immediately dissolved in 1:1 Et₂O-EtOH (4 mL), cooled to 0° C. and treated with solid NaBH₄ (~4 mg, 0.09 mmol). After stirring for 15 min, saturated aqueous NH₄Cl was cautiously added and the mixture was extracted

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with EtOAc (3 \times). The combined extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol 411 (45.6 mg, 80% for 3 steps).



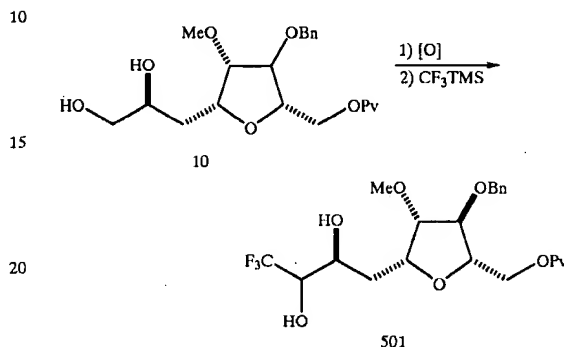
412A and 412B Alcohol 411 (120 mg, 0.196 mmol) and MPMOTCI (0.17 g, 0.59 mmol) were combined, azeotroped from toluene (3 \times) and dried under high vacuum for 1 h. CH₂Cl₂ (9 mL) was added and the mixture was cooled to 0° C. BF₃·OEt₂ (0.016 M in CH₂Cl₂, 125 μ L, 0.002 mmol) was added dropwise and after stirring for 20 min, the reaction was quenched with saturated aqueous NH₄Cl. The mixture was extracted with CH₂Cl₂ (3 \times) and the combined extracts were washed with brine, dried over Na₂SO₄, concentrated and purified by preparative TLC (20% EtOAc-hexanes) to provide the intermediate MPM ether which contained some close-running impurities. This material was immediately dissolved in Et₂O (10 mL) and treated with LAH (1M in THF, 300 μ L, 0.300 mmol) at 0° C. After 10 min, H₂O and 1 M NaOH were added, and after stirring for 10 min at rt, the mixture was filtered through Celite, concentrated and purified by preparative TLC (35% EtOAc-hexanes) to provide 412A (49 mg, 39% for 2 steps) as a single C34 isomer and 412B (46 mg, 36% for 2 steps) as a ~9:1 mixture of C34 isomers.



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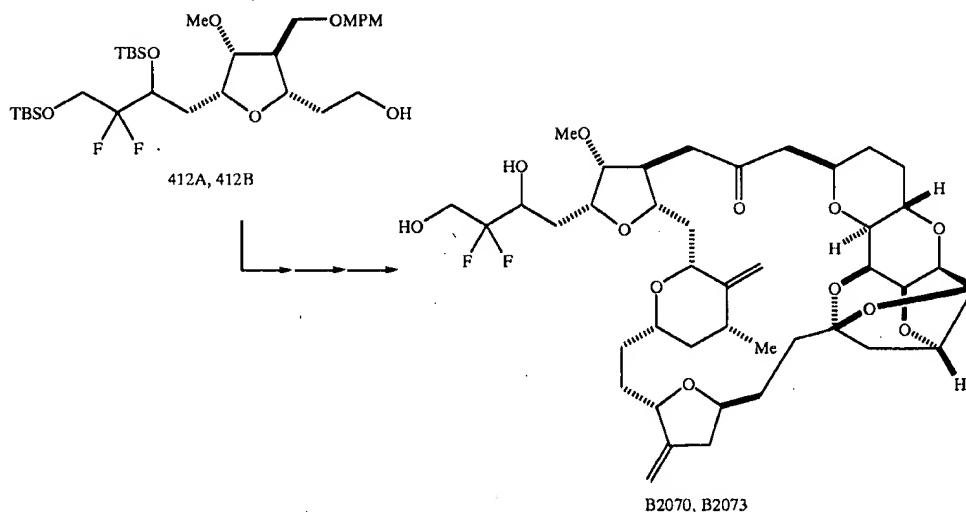
B2070 and B2073 In a manner similar to that described in Schemes 4 and 6 for the synthesis of B1794, intermediates 412A and 412B were converted to B2070 and B2073, respectively. For B2070: HRMS (FAB): calcd for C₄₁H₅₈F₂O₁₂+Na 803.3794. Found: 803.3801. For B2073: HRMS (FAB): calcd for C₄₁H₅₈F₂O₁₂+Na 803.3793. Found: 803.3781

Synthesis of B1963:



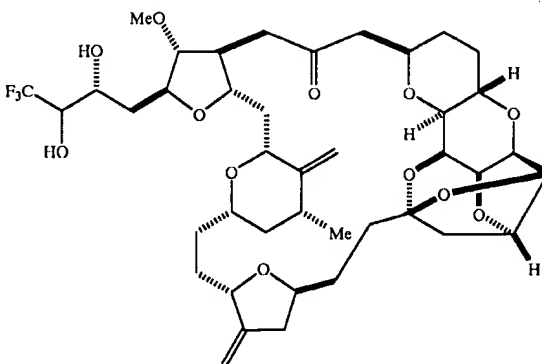
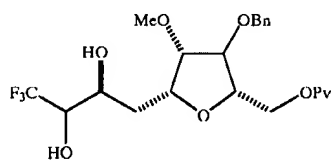
Diol 501 (64) Saturated aqueous NaHCO₃ (21 mL) and KBr (89 mg, 0.75 mmol) were added to a solution of diol 10 (1.35 g, 3.4 mmol) in CH₂Cl₂ (34 mL). The mixture was cooled to 0° C., and 4-methoxy-2,2,6,6-tetramethyl-1-piperidinyloxy (0.05 M in CH₂Cl₂, 7.45 mL, 0.37 mmol) and NaOCl (0.07 M in H₂O, 5.6 mL, 0.39 mmol) were sequentially added. After 1 h, the reaction mixture was quenched with saturated aqueous Na₂S₂O₃, diluted with saturated aqueous NaHCO₃ and extracted with CH₂Cl₂ (3 \times). The combined extracts were dried over Na₂SO₄, concentrated and dissolved in TMF (21 mL).

After cooling to 0° C., CF₃TMS (1.5 g, 10.5 mmol) and TBAF (0.1 M in THF, 680 μ L, 0.068 mmol) were sequentially added. After stirring for 40 min, additional TBAF (1 M in THF, 8.3 mL, 8.3 mmol) was added. After 30 min, the reaction was quenched with H₂O and extracted with EtOAc (3 \times). The combined organic extracts were washed with



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brine, dried over Na_2SO_4 , concentrated and purified by flash chromatography (30%, 40%, 50% EtOAc-hexanes followed by EtOAc) to afford a 2:1 mixture of diols (553 mg, 35%). Separation by MPLC (1.5% MeOH— CH_2Cl_2) gave the major, more polar isomer 501 (64) (340 mg, 22%) and the minor, less polar isomer (152 mg, 10%).

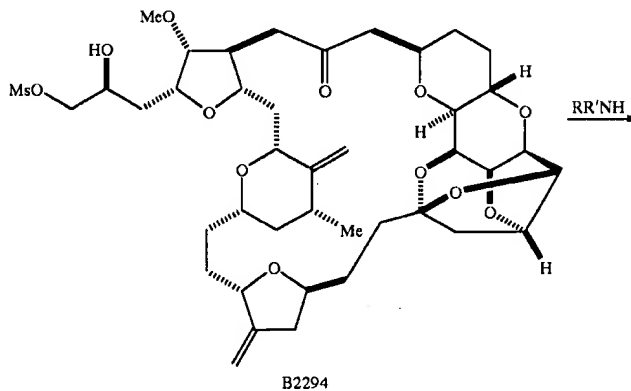


B1963

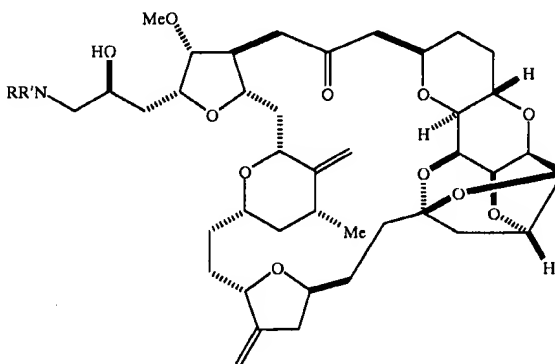
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B1963 In a manner similar to that described in Schemes 4 and 6 for the synthesis of B1794, intermediate 501 was converted to B1963.

30 Synthesis of B2320 and Related Analogs



B2294

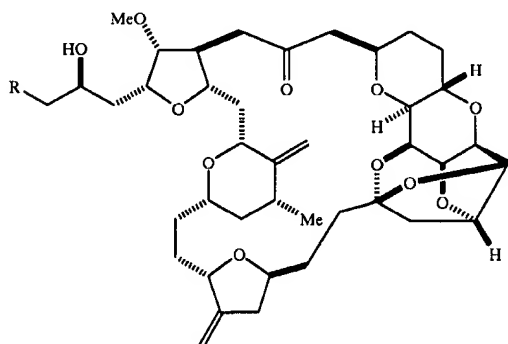


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These compounds are made by treating B2294 with an appropriate amine in a solvent such as methanol for a period of a few hours to several days. Progress of the reaction may be monitored by thin layer chromatography. A standard work-up procedure, well known to those of skill in the art, provides the desired compounds. The procedure below is to prepare ER803868; however this procedure is general and can be used to prepare any desired analog.

Synthesis of ER803868

To a solution of B2294, 1.2 mg, in methanol, 0.5 mL, was added morpholine, 0.012 mL. The mixture was stirred for 10 days with additional morpholine, 0.012 mL, being added on days 1, 2, 3, 4 and 8. The mixture was then chromatographed to give 1.4 mg of the desired compound.



B2320	R = N,N-dimethylamino
B2330	R = N-isopropylamino
B2336	R = N-methylamino
B2339	R = N-t-butylamino
B2417	R = N-2-hydroxyethylamino
B2418	R = N-piperazinyl
B2489	R = N,N-bis-(2-hydroxyethyl)amino
B2490	R = N-1,3-dihydroxy-2-propylamino
B2491	R = N-benzylamino
ER803834	R = N-piperidinyl
ER803835	R = N-pyrrolidinyl
ER803836	R = N-3-(R)-hydroxypyrrolidinyl
ER803843	R = N-homopiperidinyl
ER803845	R = N-para-methoxybenzylamino
ER803846	R = N-phenethylamino
ER803851	R = N-2-(S-hydroxymethyl)pyrrolidinyl
ER803852	R = N-2-(R-hydroxymethyl)pyrrolidinyl
ER803868	R = N-morpholinyl
ER803869	R = N-ethylamino
ER803870	R = N-imidazolyl
ER803883	R = N,N-diethylamino
ER803884	R = N-para-chlorobenzylamino

D. Pharmacological Activity

Many of the individually disclosed drugs were tested for in vitro and in vivo activity (see Table 1, below). Screening methods included a standard in vitro cell growth inhibition assay using DLD-1 human colon cancer cells (ATCC accession number CCL 221) in a 96-well microtiter plate format (Finlay, G. J. et al Analytical Biochemistry 139:272-277, 1984), a U937 (ATCC accession number CRL 1593) mitotic block reversibility assay (described below), and in some cases, a LOX human melanoma tumor xenograft in vivo growth inhibition assay (see Table 1). Chemical stability to esterase degradation was also examined.

U937 Mitotic Block Reversibility Assay

U937 human histiocytic lymphoma cells were added to 75 cm² tissue culture flasks as 2.5x10⁶ cells in 22.5 mL of

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RPMI Medium 1640 containing 10% Fetal Bovine Serum. Cells were allowed to adapt to the culture during 36 h of incubation at 37° C. in a humidified atmosphere containing 5% CO₂. Each test drug was then added to a flask as 2.5 mL of 10x final concentration. Final concentrations achieved were 0.1-1000 nM, in half log-increments, for a total of 10 concentration steps including a drug-free control flask which received 2.5 mL of media. Cells were incubated with drug for 12 h pretreatment period at 37° C. in a humidified atmosphere containing 5% CO₂.

The contents were removed from each flask and centrifuged at 300xg for 10 min at room temperature, after which drug-containing media was removed from cell pellet. Cells were resuspended in 25 mL of warm drug-free media and centrifuged at 300xg for 10 min at room temperature. After removing media from cell pellet, cells were resuspended in 35 mL of warm drug-free media, transferred to fresh flasks, and a 10 mL sample of cells immediately removed from each flask, immediately processed as described below and stored for later cell cycle analysis (0 hours of drug washout).

Incubation of the remaining 25 mL of cells continued in drug-free media for another 10 h. A 10 mL sample of cells was removed from each flask, immediately processed and stored for later cell cycle analysis (10 hours of drug washout) and 10 mL fresh replacement media was added to each incubation flask. Incubation of cells in drug-free media continued for 5 days. At day two, 20 mL of media and cells was removed from each flask and replaced with 20 mL fresh media. Viability of cells was quantified after 5 days by trypan blue exclusion techniques using hemacytometer counting.

Cells were processed for cell cycle analysis using modifications of the method published in Becton Dickinson Immunocytometry Systems source book section 1.11 (Preparation of Alcohol-Fixed Whole Cells From Suspensions For DNA Analysis). Briefly, each 10 mL sample of cells removed from the flasks at 0 and 10 hours of drug washout was separately centrifuged at 300xg for 10 min. After removing the media from the cell pellet, cells were resuspended in 3 mL cold saline. Seven milliliters cold 100% ethanol was slowly added with vigorous vortexing. Ethanol treated cell samples from 0 hour and 10 hour periods of compound washout were stored overnight at 4° C. Ethanol treated cells were centrifuged 300xg for 10 min, ethanol removed and cells then washed in 10 mL Phosphate Buffered Saline (PBS). Cells were resuspended in 0.5 mL of 0.2 mg/mL Ribonuclease A (Sigma No. R-5503) in PBS and incubated in 37° C. water bath for 30 min.

Cells were transferred to appropriate flow cytometry tubes and 0.5 mL of 10 mg/mL propidium iodide (PI) (Sigma No. P4170) in PBS was added to each tube. Cells were incubated with PI at room temperature in the dark for at least 15 min prior to analysis with a flow cytometer (Becton Dickinson FACScan flow cytometer or equivalent). Cells should be analyzed within an hour and kept in the dark at 4° C. until ready. Cell cycle analysis was performed on 0 hour and 10 hour cells using flow cytometric measurement of the intensity of cellular fluorescence. The intensity of propidium iodide fluorescence for each cell was measured on a linear amplification scale with doublet events ignored using doublet discrimination. The results obtained from analyzing 15,000 cells were presented as a histogram with increasing fluorescence intensity on the x-axis and the number of cells at a particular intensity level on the y-axis.

The intensity of PI staining is dependent on the amount of DNA in the cell so it is possible to identify cells in various

phases of the cell cycle, such as cells that have not yet synthesized DNA since the last mitosis (G₁ phase), cells that are in intermediate stages of DNA synthesis (S phase), and cells that have doubled their complement of DNA and are ready to divide (G₂ phase). Cells that are blocked in the mitosis phase of the cell cycle also have double the amount of DNA compared to G₁ phase cells. If all cells are blocked in mitosis there are no G₁ phase cells, but if the block is removed when compound is removed, cells complete mitosis and reappear in the G₁ phase. The number of cells so reappearing in the G₁ or S phase is thus a measure of the number of cells which have recently completed mitosis. For each sample at 0 and 10 hours after compound removal, the percentage of cells completing mitosis was quantified (as the number of cells reappearing in the G₁ phase) and plotted as a function of the initial concentration of compound used during the 12 hour pretreatment period. The percentage of cells still viable 5 days after drug washout was superimposed on the same graph. A ratio can be determined between the compound concentration required to completely block all cells in mitosis at 0 hour and the concentration required to maintain the block 10 hours after compound removal. This was taken as a measure of a compound's reversibility, with ratios close to or equal to one indicating likely potent in vivo anti-tumor compounds.

TABLE 1

<u>In Vitro Inhibition and Reversibility Data</u>					
Com- pound	DLD-1*		Complete Mitotic Block		
	mean IC50, nm	SE	0 hour, nM**	10 hour, nM‡	Reversibility Ratio
B1793	0.93	0.04	3	44	14.7
B1794	12.20	0.72			
B1918	1.27	0.12			
B1920	2.00	0.15			
B1921	24.00	1.15			
B1922	0.53	0.01	3	30	10.0
B1930	0.87	0.03			
B1933	0.79	0.16			
B1934	1.05	0.21	3	30	10.0
B1939	19.34	2.36	12	12	1.0
B1940	5.43	0.62			
B1942	0.60	0.03	3	30	10.0
B1963	0.56	0.04	3	20	6.7
B1973	1.15	0.24			
B1984	1.01	0.15			
B1987	1.82	0.21			
B1988	2.67	1.02			
B1990	1.30	0.06			
B1991	0.69	0.03			
B1992	0.86	0.07			
B1998	1.23	0.13			
B2003	1.21	0.12			
B2004	0.63	0.04			
B2008	2.63	0.63			
B2010	0.71	0.12			
B2011	1.81	0.52			
B2013	0.49	0.07	2	30	15.0
B2014	0.87	0.09			
B2015	2.78	0.23			
B2016	0.66	0.06			
B2019	0.82	0.07			
B2034	0.74	0.03			
B2035	0.76	0.09			
B2037	0.66	0.11			
B2039	0.91	0.08			
B2042	1.93	0.11	3	100	33.3
B2043	1.70	0.06			
B2070	0.64	0.09	3	30	10.0
B2073	0.89	0.15			
B2086	11.17	1.96			

TABLE 1-continued

<u>In Vitro Inhibition and Reversibility Data</u>					
Com- pound	DLD-1*		Complete Mitotic Block		
	mean IC50, nm	SE	0 hour, nM**	10 hour, nM‡	Reversibility Ratio
B2088	1.23	0.12			
B2090	0.52	0.04	2	10	5.0
B2091	1.36	0.07	3	30	10.0
B2102	3.47	0.22			
B2136	5.23	1.04	3	3	1.0
B2294	0.80	0.01			
B2320	1.20	0.17	1	10	10.0
B2330	4.40	0.42	7	7	1.0
B2336	3.33	0.09	10	10	1.0
B2339	4.30	0.21	3	3	1.0
B2417	12.67	0.33	10	10	1.0
B2418	3.63	0.17	10	10	1.0
B2489	14.67	2.03	10	10	1.0
B2490	35.67	3.33	100	100	1.0
B2491	0.92	0.14	2	10	6.7
ER803834	0.47	0.08	1	10	10.0
ER803835	15.33	0.33	10	10	1.0
ER803836	1.97	0.12	3	10	3.3
ER803843	0.49	0.05	1	10	10.0
ER803845	1.50	0.20	3	10	3.3
ER803846	1.16	0.10	1	10	10.0
ER803851	3.33	0.26	3	3	1.0
ER803852	3.03	0.50	3	10	3.3
ER803868	0.43	0.03	3	10	3.3
ER803869	4.13	0.64	3	3	1.0
ER803870	1.27	0.12	3	30	10.0
ER803883	1.02	0.04	1	10	10.0
ER803884	0.59	0.03	1	10	10.0

* = in vitro cell growth inhibition

** = before washout

‡ = after washout

The invention also features a method for identifying an agent that induces a sustained mitotic block in a cell after transient exposure of the cell to the agent. The invention features determining the relative reversibility of the test compound by relating the measurement of step (d) and the measurement of step (f), as described below. This determination may be a ratio, or an arithmetic difference, for example. In one aspect, the method includes:

(a) incubating a first cell sample with a predetermined concentration of a test compound for a time interval between that sufficient to empty the G₁ population and that equivalent to one cell cycle (e.g., typically, 8–16 hours, or about 12 hours);

(b) substantially separating the test compound from said first cell sample (e.g. by washing or changing media);

(c) incubating said first sample in media free of the test compound for a time interval sufficient to allow at least 80% (e.g., 85%, 90%, and preferably 95%, 98%, or 99%) of the cells released from the mitotic block induced by a highly reversible mitotic inhibitor to complete mitosis and return to the G₁ phase (e.g., typically 6–14 hours, or about 10 hours after separation step (b)); and

(d) measuring the percentage of transiently-exposed cells from step (c) that have completed mitosis and returned to the G₁ phase (e.g., measuring a cell cycle marker, such as DNA-dependent PI fluorescence).

One aspect of this screening method include the further steps of:

(e) incubating a second sample of cells with a concentration of the test compound less than or equal to that used in step (a) for a time interval between that sufficient to empty the G₁ population and that equivalent to one cell cycle;

(f) measuring the percentage of cells from step (e) that have completed mitosis and have returned to the G₁ phase; and

(g) determining a reversibility ratio of the test compound.

In one embodiment of the method, the first and second cell samples are suspension culture cells selected from, for example, human leukemia, human lymphoma, murine leukemia, and murine lymphoma cells. The first and second cell samples may be incubated simultaneously (steps (a) and (e)) or in separate portions. Other embodiments further include before step (a), the step (i) of estimating a desirable time interval for incubating said first cell sample with a reversible mitotic blocking agent (or, alternatively, said test compound) to provide a satisfactory majority of cells collected at mitotic block; and wherein the incubation of step (a) is for the time interval estimated in step (i). Another embodiment of the method further includes before step (c), the step (ii) of estimating a desirable time interval for the test compound-free incubation of step (c), said step (ii) comprising determining the time interval after which at least 80% of the cells pretreated with a highly reversible antimitotic agent complete mitosis and reenter G₁ phase; and wherein the incubation of step (c) is for the time interval determined in step (ii). Another embodiment of the method utilizes non-suspension culture cells from, for example, adherent human or murine cancer cells, harvested by any suitable means for detaching them from tissue culture flasks.

One aspect of the method further includes repeating steps (a)–(f) using a range of relative concentrations of test compound to determine what two substantially minimum concentrations of the test compound provide substantially complete mitotic block in step (d) and in step (f), respectively. The ratio of these minimum sufficient concentrations is an index of reversibility (see detailed U937 protocol for preparation of exemplary dose-response curves). These concentrations may be determined by extrapolating curves of the percentage of cells (from steps (d) and (f)) as a function of concentration (e.g., by testing only a few concentrations, such as 3 or fewer), or by empirically testing a full range of concentrations.

The above methods are useful for identifying an agent (test compound) that inhibits mitosis, for identifying a mitotic blocking agent which substantially retained its mitosis blocking effectiveness after its removal, and for predicting, for example, the IC₅₀ or the IC₉₅ of a mitotic blocking agent. When compared with relatively reversible antimitotic agents, substantially irreversible antimitotic agents, in other words, agents which continue to block mitosis in a cell which has been only transiently exposed to the agent, are likely to be more effective in vivo where natural processes, including multi-drug resistance (MDR) pumps and metabolic or other degradative pathways, prevent prolonged exposure. The effectiveness of relatively reversible antimitotic agents may depend upon a period of sustained exposure.

In view of the cost of developing pharmaceuticals, the economic advantages of determining reversibility ratios, as described above, are considerable. The above methods can be used, for example, to predict whether a test compound with good in vitro activity will be effective in vivo, such as in a clinical trial. Relatively reversible agents would not be expected to perform as well as irreversible agents. This is shown, for example, by contrasting the data for two known compounds, the relatively irreversible antimitotic agent vincristine and the highly reversible antimitotic agent vinblastine.

TABLE 2

Reversibility Characteristics of Vinblastine and Vincristine

Compound	Drug concentration required for complete mitotic block, nM		Reversibility ratio	Interpretation
	0 hour (before washout)	10 hour (after washout)		
Vinblastine	10	600	60	Highly Reversible
Vincristine	10	10	1	Irreversible

Analyses of the antimitotic drugs vinblastine and vincristine in the U937 Mitotic Block Reversibility Assay indicate that despite identical potencies to induce initial mitotic blocks (0 hour values), the abilities of the two drugs to induce mitotic blocks which are sustained 10 hour after drug washout (10 hour values) are very different: vincristine induces irreversible mitotic blocks, while those induced by vinblastine are highly reversible.

Analyses of in vivo anticancer activities of the antimitotic drugs vinblastine and vincristine against COLO 205 human colon cancer xenografts grown sub-cutaneously in immunocompromised (nude) mice indicate that at equivalent doses of 1 mg/kg, vincristine shows substantial cancer growth inhibitory activity while vinblastine is inactive. At the lower dose of 0.3 mg/kg, vincristine still produces moderate growth inhibition, while vinblastine is again inactive. The greater in vivo activity of vincristine correlates with its irreversibility relative to vinblastine's high reversibility.

E. Use

The disclosed compounds have pharmacological activity, including anti-tumor and anti-mitotic activity as demonstrated in section D above. Examples of tumors include melanoma, fibrosarcoma, monocytic leukemia, colon carcinoma, ovarian carcinoma, breast carcinoma, osteosarcoma, prostate carcinoma, lung carcinoma and ras-transformed fibroblasts.

The invention features pharmaceutical compositions which include a compound of formula (I) and a pharmaceutically-acceptable carrier. Compositions can also include a combination of disclosed compounds, or a combination of one or more disclosed compounds and other pharmaceutically-active agents, such as an anti-tumor agent, an immune-stimulating agent, an interferon, a cytokine, an anti-MDR agent or an anti-angiogenesis agent. Compositions can be formulated for oral, topical, parenteral, intravenous, or intramuscular administration, or administration by injection or inhalation. Formulations can also be prepared for controlled-release, including transdermal patches.

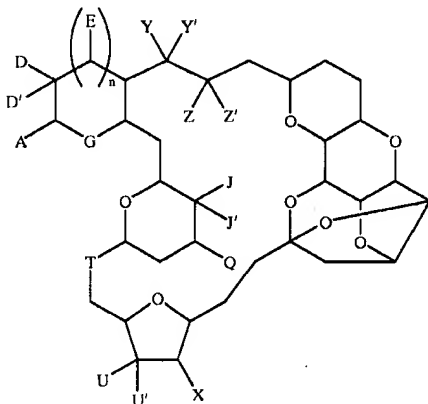
A method for inhibiting tumor growth in a patient includes the step of administering to the patient an effective, anti-tumor amount of a disclosed compound or composition. The invention also contemplates combination therapies, including methods of co-administering a compound of formula (I) before, during, or after administering another pharmaceutically active agent. The methods of administration may be the same or different. Inhibition of tumor growth includes a growth of the cell or tissue exposed to the test compound that is at least 20% less, and preferably 30%, 50%, or 75% less than the growth of the control (absence of known inhibitor or test compound).

Other Embodiments

The essential features of the invention can be easily discerned from the above description and the claims below. Based on the entire disclosure, variations of the disclosed compounds and methods of the invention described can be designed and adapted without departing from the spirit and scope of the claims and the disclosure. References and publications described herein are hereby incorporated in their entirety.

What is claimed is:

1. A compound having the formula:



wherein A is a C₁₋₆ saturated or C₂₋₆ unsaturated hydrocarbon skeleton, said skeleton being unsubstituted or having between 1 and 10 substituents, inclusive, independently selected from cyano, halo, azido, oxo, and Q₁;

each Q₁ is independently selected from OR₁, SR₁, SO₂R₁, OSO₂R₁, NR₂R₁, NR₂(CO)R₁, NR₂(CO)(CO)R₁, NR₄(CO)NR₂R₁, NR₂(CO)OR₁, (CO)OR₁, O(CO)R₁, (CO)NR₂R₁, and O(CO)NR₂R₁;

each of R₁, R₂, R₄, R₅, and R₆ is independently selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₁₋₆ hydroxyalkyl, C₁₋₆ aminoalkyl, C₆₋₁₀ aryl, C₆₋₁₀ haloaryl, C₆₋₁₀ hydroxyaryl, C₁₋₃ alkoxy-C₆ aryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ aryl, C₆₋₁₀ haloaryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ haloaryl, (C₁₋₃ alkoxy-C₆ aryl)-C₁₋₃ alkyl, C₂₋₉ heterocyclic radical, C₂₋₉ heterocyclic radical-C₁₋₆ alkyl, C₂₋₉ heteroaryl, and C₂₋₉ heteroaryl-C₁₋₆ alkyl;

each of D and D' is independently selected from R₃ and OR₃, wherein R₃ is H, C₁₋₃ alkyl, or C₁₋₃ haloalkyl; n is 0 or 1;

E is R₅ or OR₅;

G is O, S, CH₂, or NR₆;

each of J and J' is independently H, C₁₋₆ alkoxy, or C₁₋₆ alkyl; or J and J' taken together are =CH₂ or —O—(straight or branched C₁₋₅alkylene)—O—;

Q is C₁₋₃ alkyl;

T is ethylene or ethenylene, optionally substituted with (CO)OR₇, where R₇ is H or C₁₋₆ alkyl;

each of U and U' is independently H, C₁₋₆ alkoxy, or C₁₋₆ alkyl; or U and U' taken together are =CH₂ or —O—(straight or branched C₁₋₅alkylene)—O—;

X is H or C₁₋₆ alkoxy;

each of Y and Y' is independently H or C₁₋₆ alkoxy; or Y and Y' taken together are =O, =CH₂, or —O—(straight or branched C₁₋₅alkylene)—O—; and

each of Z and Z' is independently H or C₁₋₆ alkoxy; or Z and Z' taken together are =O, =CH₂, or —O—(straight or branched C₁₋₅alkylene)—O—;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein n is 0.

3. The compound of claim 1, wherein each of D and D' is independently selected from R₃, C₁₋₃ alkoxy, and C₁₋₃ haloalkoxy.

4. The compound of claim 1, wherein R₅ is selected from H, C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₁₋₆ hydroxyalkyl, C₁₋₆ aminoalkyl, C₆₋₁₀ aryl, C₆₋₁₀ haloaryl, C₆₋₁₀ hydroxyaryl, C₁₋₃ alkoxy-C₆ aryl, C₆₋₁₀ aryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ aryl, C₆₋₁₀ haloaryl-C₁₋₆ alkyl, C₁₋₆ alkyl-C₆₋₁₀ haloaryl, (C₁₋₃ alkoxy-C₆ aryl)-C₁₋₃ alkyl, C₂₋₉ heterocyclic radical, C₂₋₉ heterocyclic radical-C₁₋₆ alkyl, C₂₋₉ heteroaryl, and C₂₋₉ heteroaryl-C₁₋₆ alkyl.

5. The compound of claim 1, wherein A comprises a C₁₋₆ saturated or C₂₋₆ unsaturated hydrocarbon skeleton, said skeleton having at least one substituent selected from cyano, halo, azido, oxo, and Q₁;

each Q₁ is independently selected from OR₁, SR₁, SO₂R₁, OSO₂R₁, NR₂R₁, NR₂(CO)R₁, and O(CO)NR₂R₁;

n is 0;

G is O;

J and J' taken together are =CH₂;

Q is methyl;

T is ethylene;

U and U' taken together are =CH₂;

X is H;

each of Y and Y' is H; and

Z and Z' taken together are =O or =CH₂.

6. The compound of claim 1, wherein each Q₁ is independently selected from OR₁, SR₁, SO₂R₁, OSO₂R₁, NH(CO)R₁, NH(CO)(CO)R₁, and O(CO)NHR₁;

each R₁ is independently selected from C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₆ aryl, C₆ haloaryl, C₁₋₃ alkoxy-C₆ aryl, C₆ aryl-C₁₋₃ alkyl, C₁₋₃ alkyl-C₆ aryl, C₆ haloaryl-C₁₋₃ alkyl, C₁₋₃ alkyl-C₆ haloaryl, (C₁₋₃ alkoxy-C₆ aryl)-C₁₋₃ alkyl, C₂₋₉ heterocyclic radical, C₂₋₉ heteroaryl, and C₂₋₉ heteroaryl-C₁₋₆ alkyl;

one of D and D' is methyl or methoxy, and the other is H; n is 0;

G is O;

J and J' taken together are =CH₂;

Q is methyl;

T is ethylene;

U and U' taken together are =CH₂;

X is H;

each of Y and Y' is H; and

Z and Z' taken together are =O.

7. The compound of claim 6, wherein A has at least one substituent selected from hydroxyl, amino, azido, halo, and oxo.

8. The compound of claim 7, wherein A comprises a saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl, amino and azido.

9. The compound of claim 8, wherein A has at least two substituents independently selected from hydroxyl, amino, and azido.

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10. The compound of claim 8, wherein A has at least two substituents independently selected from hydroxyl and amino.

11. The compound of claim 8, wherein A has at least one hydroxyl substituent and at least one amino substituent.

12. The compound of claim 8, wherein A has at least two hydroxyl substituents.

13. The compound of claim 8, wherein A comprises a C₂₋₄ hydrocarbon skeleton.

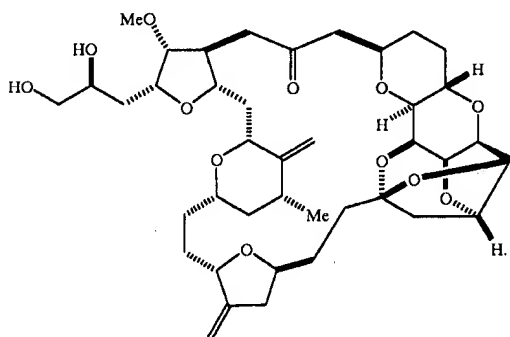
14. The compound of claim 8, wherein A comprises a C₃ hydrocarbon skeleton.

15. The compound of claim 13, wherein A has an (S)-hydroxyl on the carbon atom alpha to the carbon atom linking A to the ring containing G.

16. The compound of claim 6, wherein A comprises a C₁₋₆ saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl and cyano.

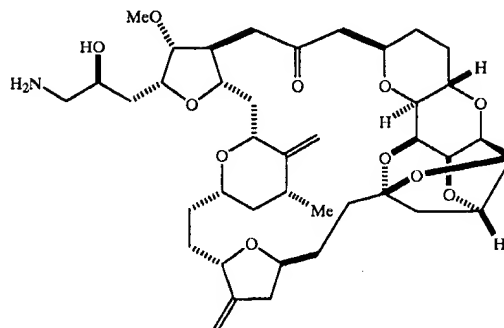
17. The compound of claim 6, wherein Q₁ is independently selected from OR₁, SR₁, SO₂R₁, and OSO₂R₁ where each R₁ is independently selected from C₁₋₆ alkyl, C₁₋₆ haloalkyl, C₆ aryl, C₆ haloaryl, C₁₋₃ alkoxy-C₆ aryl, C₆ aryl-C₁₋₃ alkyl, C₁₋₃ alkyl-C₆ aryl, C₆ haloaryl-C₁₋₃ alkyl, C₁₋₃ alkyl-C₆ haloaryl, and (C₁₋₃ alkoxy-C₆ aryl)-C₁₋₃ alkyl.

18. The compound of the following structure



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19. The compound of the following structure



and pharmaceutically acceptable salts thereof.

20. A method for identifying an agent that induces a sustained mitotic block in a cell after transient exposure of said cell to said agent, said method comprising the steps of:

- (a) incubating a first cell sample with a predetermined concentration of a test compound for a time interval between that sufficient to empty the G₁ population and that equivalent to one cell cycle;
- (b) substantially separating said test compound from said first cell sample;
- (c) incubating said first sample in media free of said test compound for a time interval sufficient to allow at least 80% of the cells released from the mitotic block induced by a highly reversible mitotic inhibitor to complete mitosis and return to the G₁ phase; and
- (d) measuring the percentage of transiently-exposed cells from step (c) that have completed mitosis and returned to the G₁ phase.

21. The method of claim 20, further comprising the steps of:

- (e) incubating a second sample of cells with a concentration of said test compound less than or equal to that used in step (a) for a time interval between that sufficient to empty the G₁ population and that equivalent to one cell cycle;
- (f) measuring the percentage of cells from step (e) that have completed mitosis and have returned to the G₁ phase; and
- (g) determining the relative reversibility of said test compound by relating the measurement of step (d) and the measurement of step (f).

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 1 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

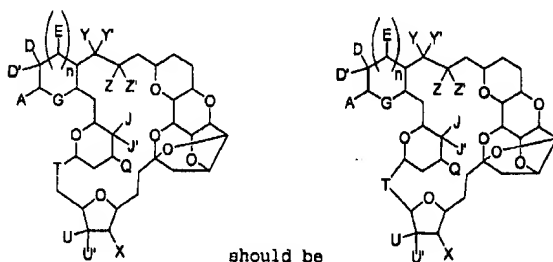
On the cover sheet: replace "Londonberry" with -- Londonderry --.

Column 1,

Line 12: replace "Lissondendryx sp." with -- Lissodendoryx sp. --.

Column 1,

Line 30: the drawing labeled "Formula (I)"



Column 2,

Line 4: replace "2-aminoethoxy" with -- 2-aminoethoxy --.

Column 2,

Line 8: replace "2,3-dihydroxy-4-perflurobutyl" with
-- 2,3-dihydroxy-4-perfluorobutyl --.

Column 3,

Line 54: replace "4H-quinolixinyI," with -- 4H-quinolizinyI --.

Column 3,

Line 55: replace "quinazolyinyI" with -- quinazolinyI --.

UNITED STATES PATENT AND TRADEMARK OFFICE
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INVENTOR(S) : Littlefield et al.

Page 2 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

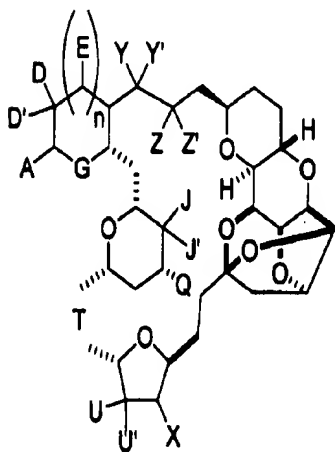
Column 4,
Line 17: replace "embodiment includes" with
-- embodiments include --.

Column 4,
Lines 22-23: replace " $\text{NR}_2(\text{CO})\text{R}_1$, $\text{NR}_2(\text{CO})\text{R}_1$ " with -- $\text{NR}_2(\text{CO})\text{R}_1$, --.

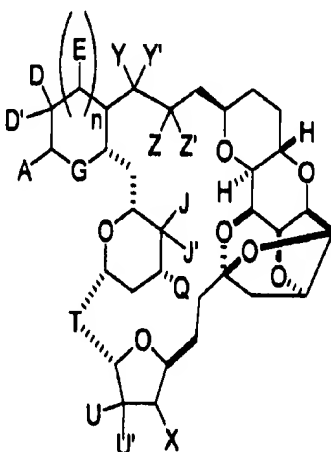
Column 4,
Line 40: replace "B13939" with -- B1939 --.

Column 4,
Line 57: replace ", B13939" with -- , B1939 --.

Column 5,
Line 20: the drawing labeled "Formula 1-A"



should be



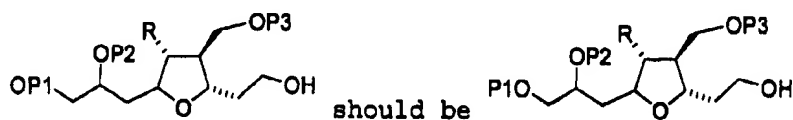
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

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APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 3 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 5,
Line 42: the drawing labeled "Formula (II)"



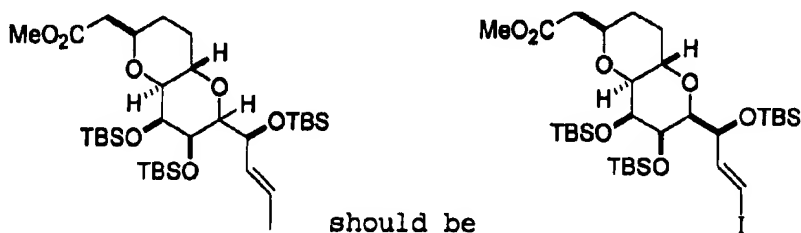
Column 5,
Line 63: replace "9-fluorenylinethyl" with -- 9-fluorenylmethyl --.

Column 6,
Line 9: replace "O-nitrobenzyl" with -- *o*-nitrobenzyl --.

Column 6,
Line 26: replace "1,1,3,3-tetra-isopropylidisiloxanylidene" with
-- 1,1,3,3-tetra-isopropylidisiloxanylidene --.

Column 6,
Line 52: replace "Scola, M." with -- Scola, P.M. --.

Column 7,
Line 1: the drawing labeled "XF3"



UNITED STATES PATENT AND TRADEMARK OFFICE
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PATENT NO. : 6,214,865 B1
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Page 4 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

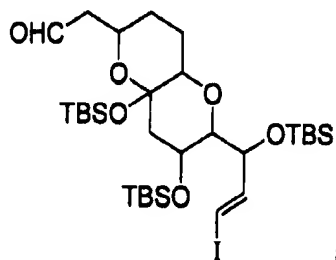
Column 7,
Line 42: replace "p-F—PhMgBr" with -- p-F-PhMgBr --.

Column 8,
Line 2: replace "furnished key fragment I" with
-- furnished key fragment F1 --.

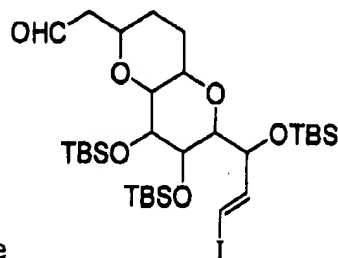
Column 8,
Line 8: replace "fluorinated key fragment I was obtained" with
-- fluorinated key fragment F1 was obtained --.

Column 8,
Line 18: replace "homoallylic alcohol 33" with
-- homoallylic alcohol 33a --.

Column 8,
Line 55: the drawing labeled "F-3"



should be



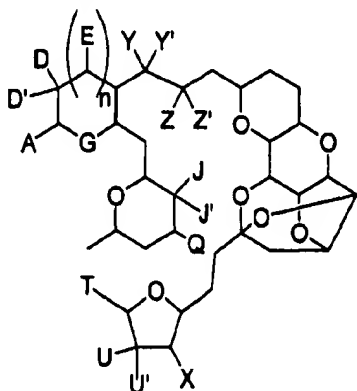
UNITED STATES PATENT AND TRADEMARK OFFICE
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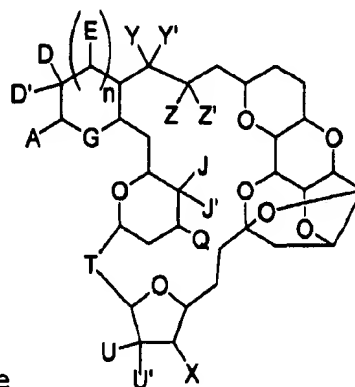
Page 5 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

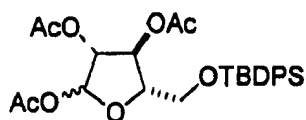
Column 9,
Line 2: the drawing labeled "4, Formula (I)"



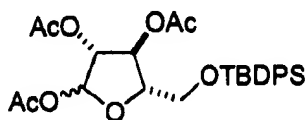
should be



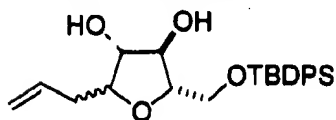
Column 9,
Line 55: the drawing labeled "2"



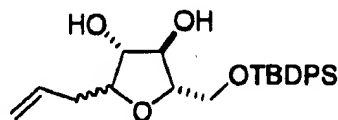
should be



Column 9,
Line 60: the drawing labeled "4a,4b"



should be



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
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INVENTOR(S) : Littlefield et al.

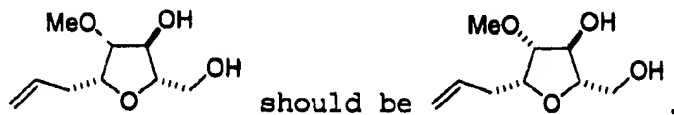
Page 6 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

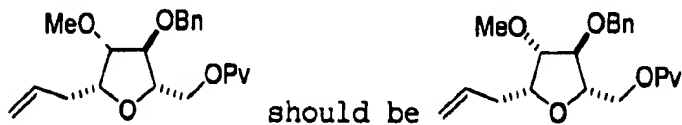
Column 9,
Line 60: the reagent

 3) MeI should be  3) MeI .

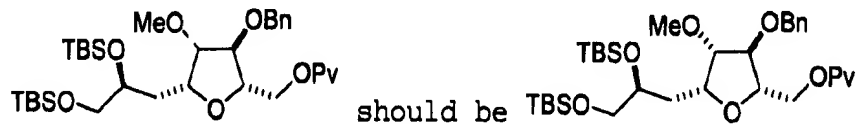
Column 10,
Line 1: the drawing labeled "7"



Column 10,
Line 10: the drawing labeled "9"



Column 10,
Line 15: the drawing labeled "11"



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
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INVENTOR(S) : Littlefield et al.

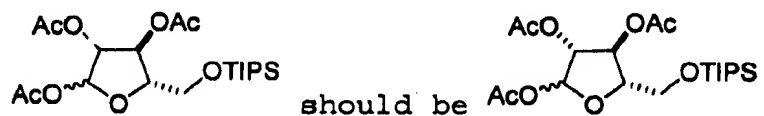
Page 7 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

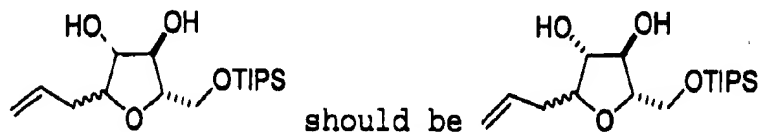
Column 10,
Line 21: the reagent

$\xrightarrow{\text{1) MPMOTCI}}$ should be $\xrightarrow{\text{1) MPMOTCI}}$.

Column 10,
Line 45: the drawing labeled "XX14"



Column 10,
Line 52: the drawing labeled "XX15"



Column 10,
Line 55: the reagent

$\xrightarrow{\text{3) MeI}}$ should be $\xrightarrow{\text{3) MeI}}$.

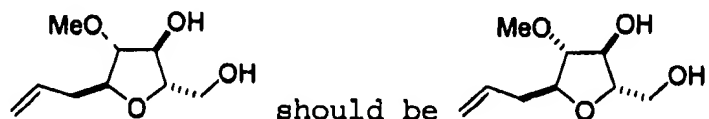
UNITED STATES PATENT AND TRADEMARK OFFICE
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DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

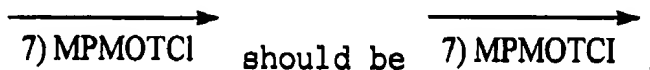
Page 8 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

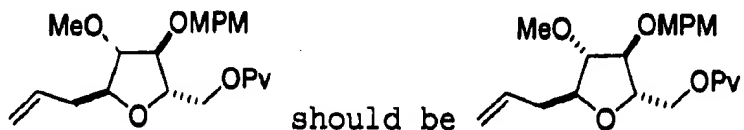
Column 10,
Line 60: the drawing labeled "XX16"



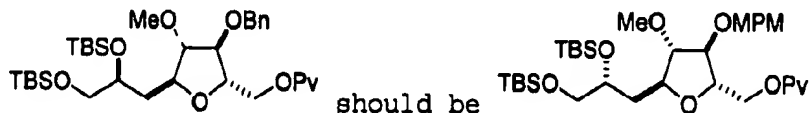
Column 10,
Line 63: the reagent



Column 11,
Line 1: the drawing labeled "XX17"



Column 11,
Line 9: the drawing labeled "XX18"



Column 11,
Line 16: the reagent



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

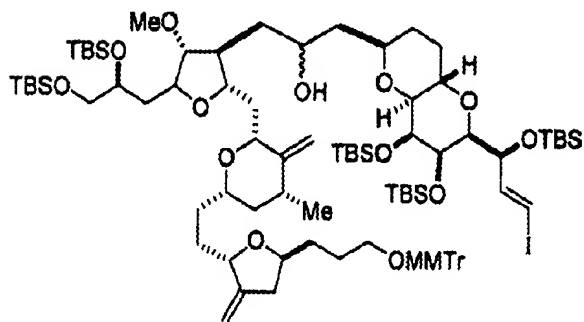
Page 9 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

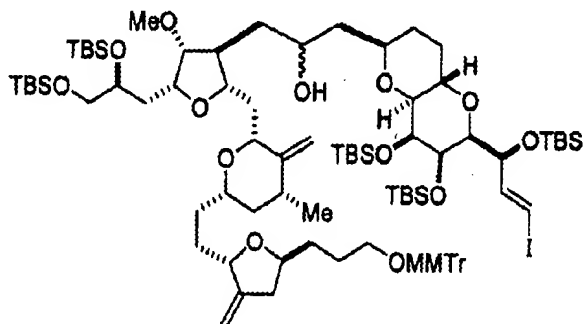
Column 12,
Line 1: the reagent

7) MPMOTCI $\xrightarrow{\quad}$ should be 7) MPMOTCI $\xrightarrow{\quad}$

Column 13,
Line 1: the drawing labeled “B2307, B2308”



should be



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

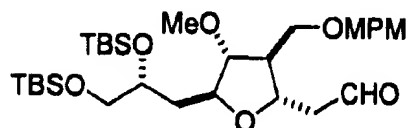
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 10 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

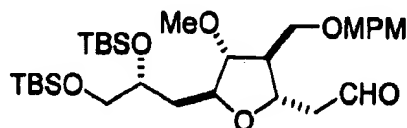
Column 13,

Line 20: the drawing labeled "X20" should be labeled -- XX20 -- as follows:



X20

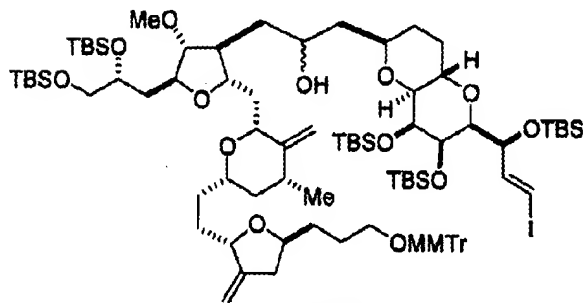
should be



XX20

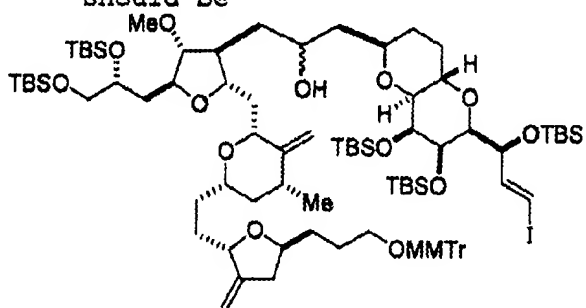
Column 15,

Line 1: the drawing labeled "BX2308" should be labeled -- BX2307, BX2308 -- as follows:



BX2308

should be



BX2307, BX2308

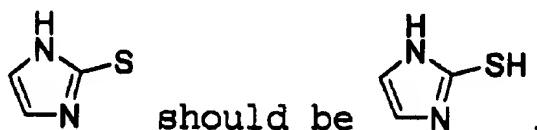
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

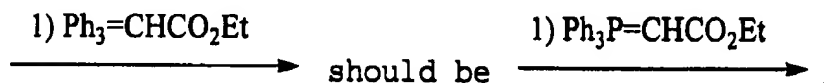
Page 11 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

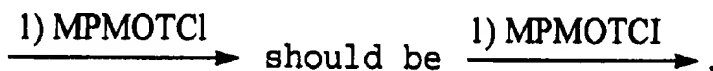
Column 21,
Line 1: the reagent



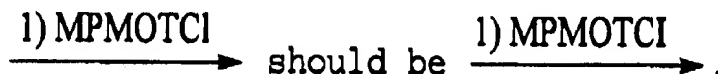
Column 21,
Line 60: the reagent



Column 22,
Line 60: the reagent



Column 23,
Line 45: the reagent



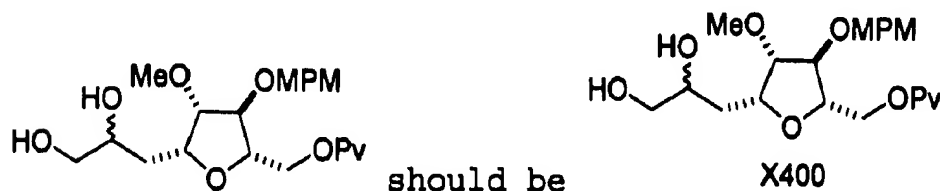
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 12 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

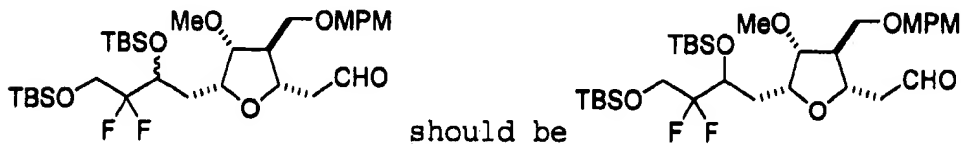
Column 24,
Line 20: the unlabeled drawing should be labeled as -- X400 -- as follows:



Column 24,
Line 45: the reagent



Column 24,
Line 55: the drawing labeled "X412" should be labeled -- X412A, X412B --.
Additionally, the drawing labeled "X412"



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 13 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 25,

Line 2: the drawing labeled "10" should be labeled -- 10a or 10b -- as follows:



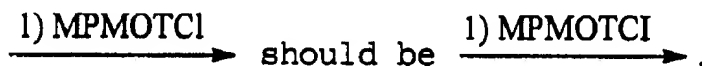
Column 25,

Line 34: the reagent



Column 26,

Line 15: the reagent



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

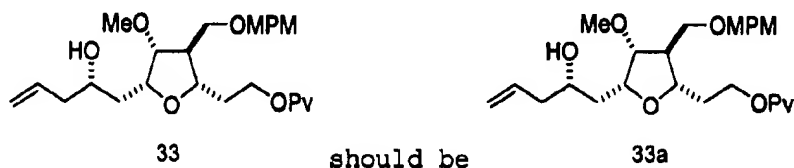
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 14 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 26,

Line 35: the drawing labeled "33" should be labeled -- 33a -- as follows:

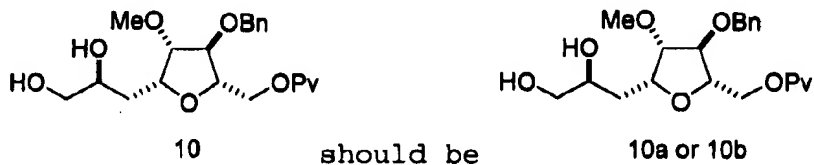


Column 29,

Line 63: replace "BF₃ · OEt₂" with -- BF₃ · OEt₂ --.

Column 32,

Line 35: the drawing labeled "10" should be labeled -- 10a or 10b -- as follows:



Column 32,

Line 39: replace "Diol 10" with -- Diol 10a or 10b --.

Column 32,

Line 44: replace "Na₂S₂O₅ · 5 H₂O" with -- Na₂S₂O₅ · 5H₂O --.

Column 32,

Line 49: replace "diol 10" with -- diol 10a or 10b --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

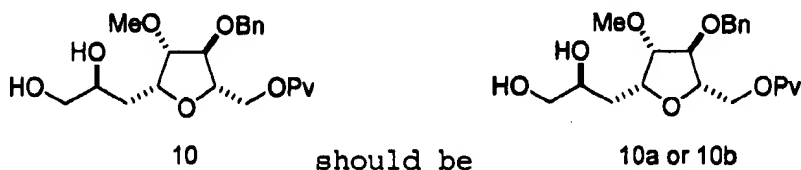
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 15 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 32,

Line 55: the drawing labeled "10" should be labeled -- 10a or 10b -- as follows:



Column 32,

Line 66: replace "diol 10" with -- diol 10a or 10b --.

Column 34,

Line 20: replace " $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$ " with -- $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ --.

Column 34,

Line 45: replace "0.021 mmol" with -- 0.021 mol --.

Column 35,

Line 5: the reagent



Column 35,

Line 18: replace " $\text{BF}_3 \cdot \text{OEt}_2$ " with -- $\text{BF}_3 \cdot \text{OEt}_2$ --.

Column 35,

Line 22: replace " $\text{BF}_3 \cdot \text{OEt}_2$ " with -- $\text{BF}_3 \cdot \text{OEt}_2$ --.

Column 36,

Line 53: replace " $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$ " with -- $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ --.

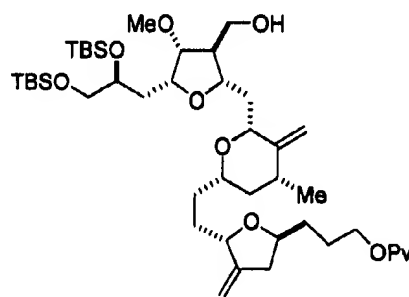
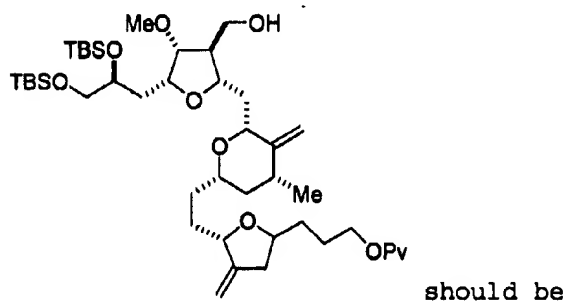
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

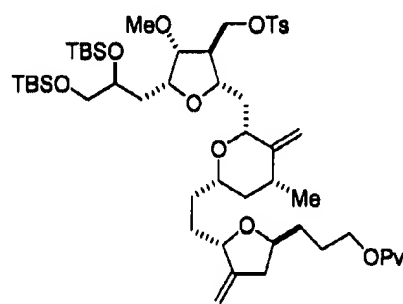
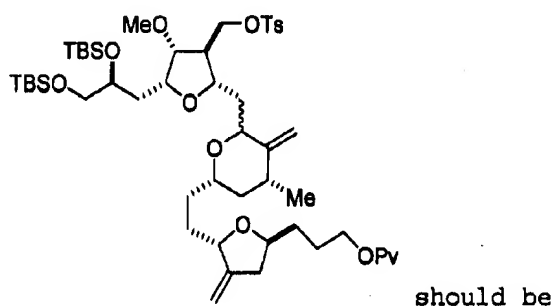
Page 16 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 39,
Line 1: the drawing labeled "B2317"



Column 39,
Line 50: the drawing labeled "B2316"



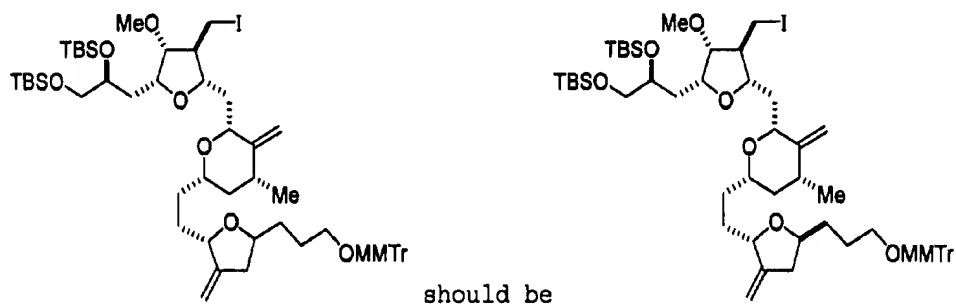
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 17 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 41,
Line 25: the drawing labeled "B2313"



Column 42,
Line 17: replace "B2308" with -- B2307 and B2308 --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

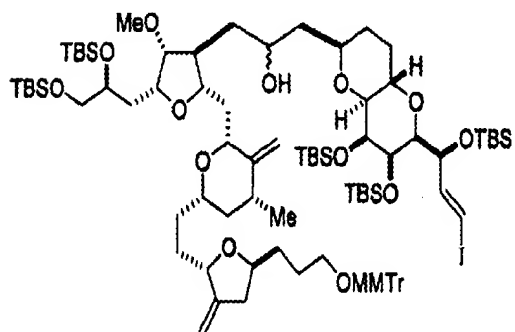
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 18 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

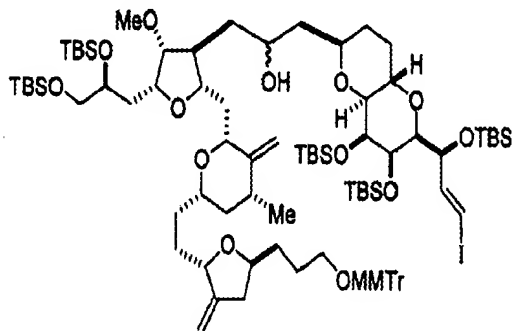
Column 42,

Line 35: the drawing labeled "B2308" should be labeled -- B2307, B2308 -- as follows:



B2308

should be



B2307, B2308

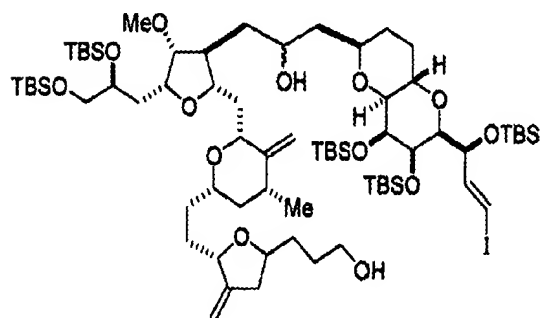
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

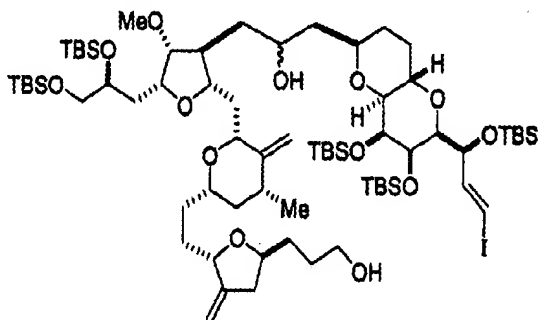
Page 19 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 42,
Line 45: the drawing labeled "B2305, B2306"



should be



Column 44,
Line 21: replace "alcohol, 2.4 g mg," with -- alcohol, 2.4 g, --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 20 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 44,

Line 22: replace "triflic anhydride" with -- methanesulfonyl anhydride --.

Column 44,

Line 24: replace "chromatrographed" with -- chromatographed --.

Column 44,

Line 61: replace "DMF, 0.06 mL," with -- DMF, 0.5 mL, --.

Column 45,

Lines 39-40: replace

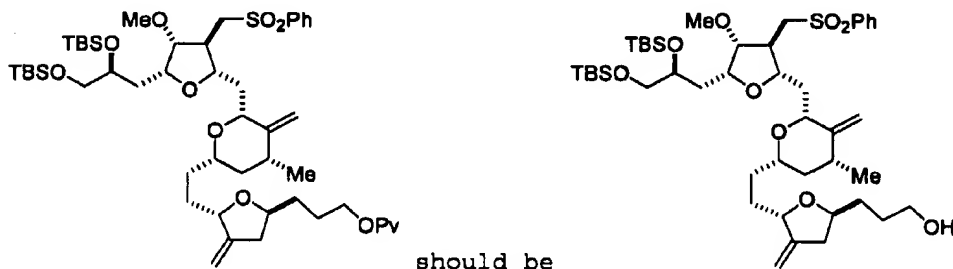
"N-methylmorpholine oxide (NMO), and then a solution of 1.02 g, tetrapropylammonium perruthenate"

with

-- N-methylmorpholine oxide (NMO) (1.02 g), and then a solution of tetrapropylammonium perruthenate --.

Column 46,

Line 30: the drawing labeled "ER804028"



Column 49,

Line 26: replace "SmI reagent, 0.05 mL, as added" with
-- SmI₂ reagent, 0.05 mL, was added --.

Column 54,

Line 21: replace (Schemes 3 and 5) with -- (Schemes 4 and 6) --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

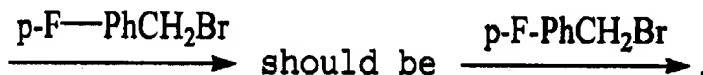
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 21 of 41

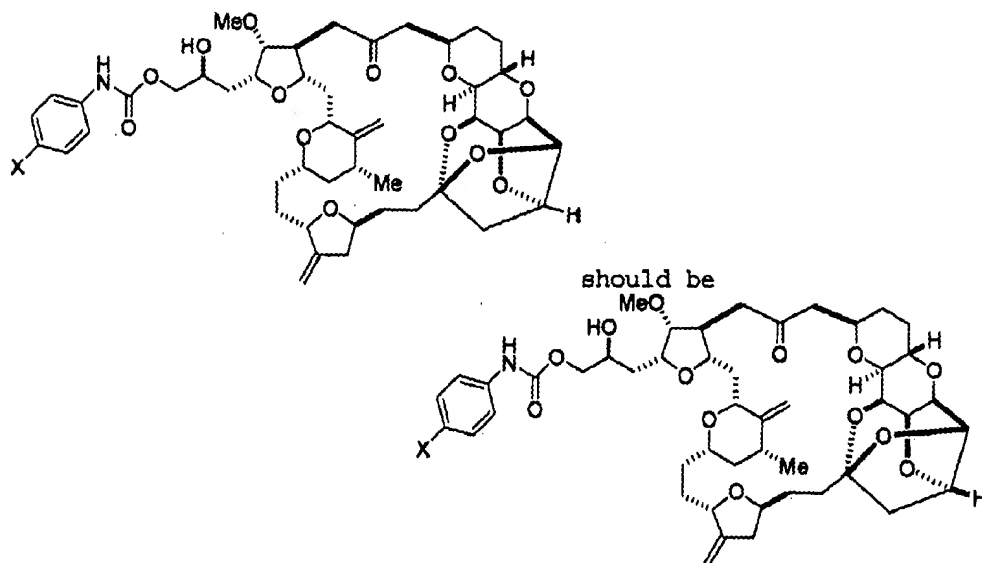
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 54,
Lines 22-23: replace (see schemes 4 and 5) with -- (see schemes 3 and 5) --.

Column 56,
Line 40: the reagent



Column 59,
Line 1: the drawing labeled "B1984, B1990, B1992"



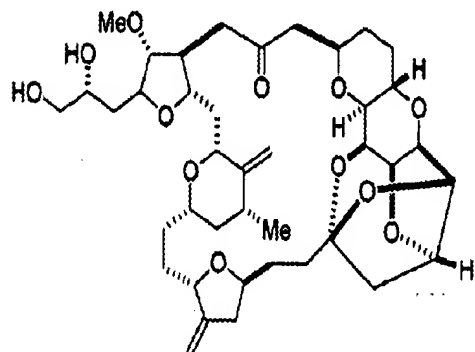
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

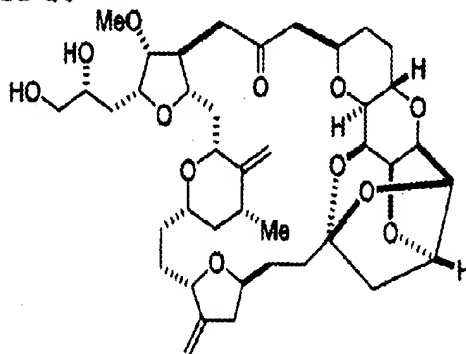
Page 22 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 60,
Line 40: The drawing labeled "B2042"



should be



Column 62,
Lines 7-8: replace "Found: 753.3969" with -- Found: 755.3969 --.

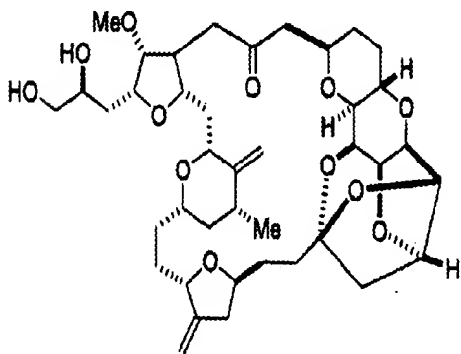
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

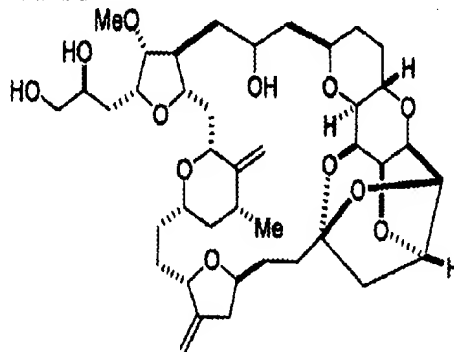
Page 23 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 62,
Line 10: the drawing labeled "B1896, B1897"



should be



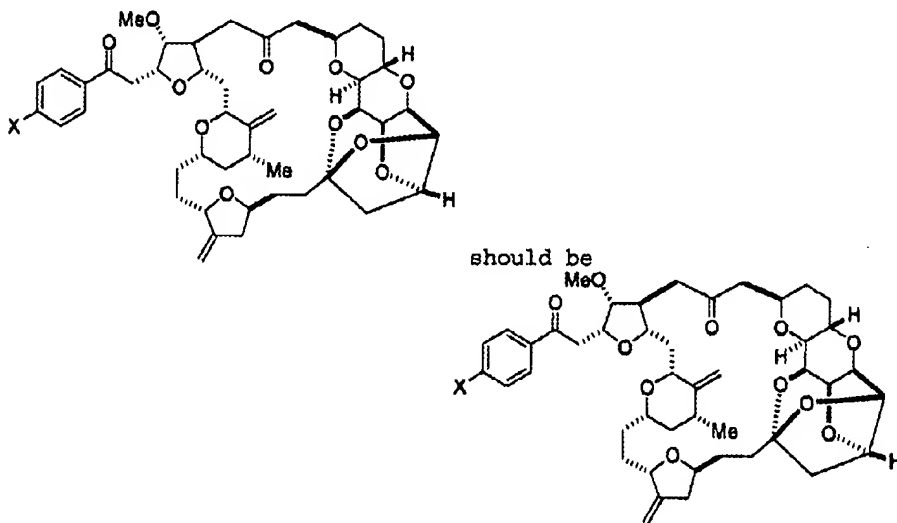
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 24 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 67,
Line 1: the drawing labeled "B2011, B2008"



Column 71,
Line 41: replace "897.3521. Found: 897.3533." with
-- 879.3521. Found: 879.3533. --.

Column 72,
Line 45: the reagent

1) TBDPSCI → should be 1) TBDPSCI → .

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 25 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 72,
Line 45: the reagent

$\xrightarrow{\quad}$ 3) DBAF should be 3) TBAF $\xrightarrow{\quad}$.

Column 78,
Line 30: replace " $C_{50}H_{64}N_2O_{12}+Na$ " with -- $C_{50}H_{64}N_2O_{13}+Na$ --.

Column 78,
Line 46: replace " $C_{49}H_{65}NO_{13}+Na$ " with -- $C_{50}H_{69}NO_{15}+Na$ --.

Column 79,
Line 25: replace "a 1:9 mixture" with -- a 9:1 mixture --.

Column 79,
Line 28: replace "Found: 884.4012" with -- Found: 882.4012 --.

Column 81,
Line 40: the reagent

$\xrightarrow{1) Ph_3=CHCO_2Et}$ should be $\xrightarrow{1) Ph_3P=CHCO_2Et}$.

Column 83,
Line 35: the reagent

$\xrightarrow{MPMOTCl}$ should be $\xrightarrow{MPMOTCI}$.

Column 83,
Line 54: replace " $BF_3 \cdot OEt_2$ " with -- $BF_3 \cdot OEt_2$ --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

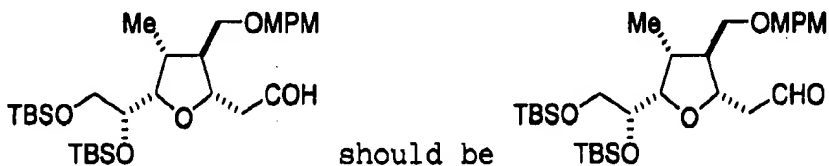
Page 26 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

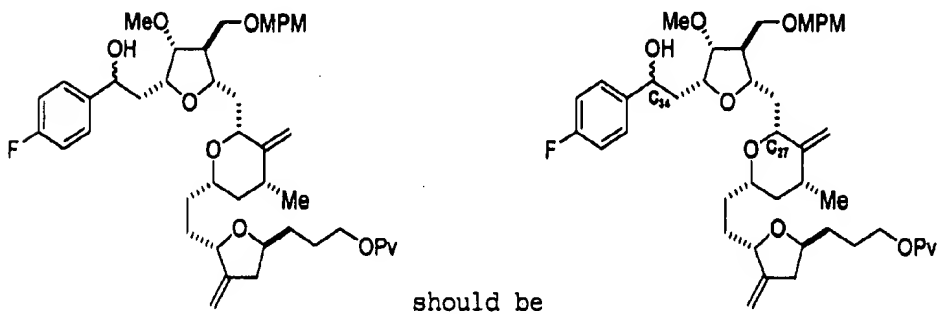
Column 84,
Line 28: replace "TsOH . H₂O" with -- TsOH • H₂O --.

Column 84,
Line 30: replace "TsOH . H₂O" with -- TsOH • H₂O --.

Column 86,
Line 1: the drawing labeled "114"



Column 90,
Line 1: the drawing labeled "203"



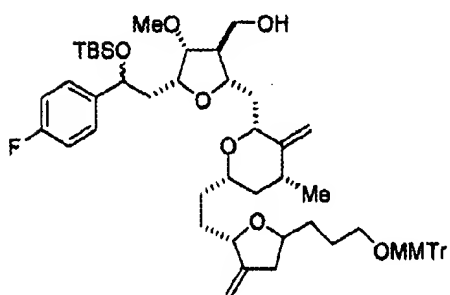
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

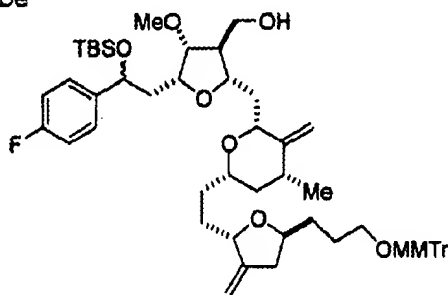
Page 27 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 93,
Line 5: the drawing labeled "207"



should be



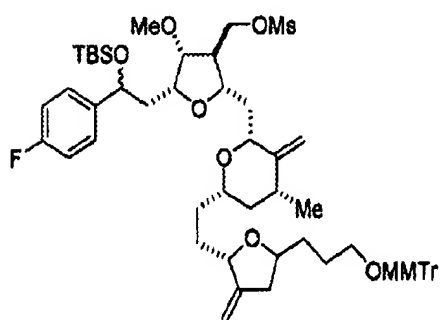
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

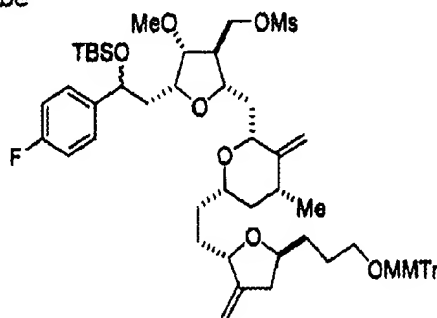
Page 28 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 93,
Line 25: the drawing labeled "208A and 208B"



should be



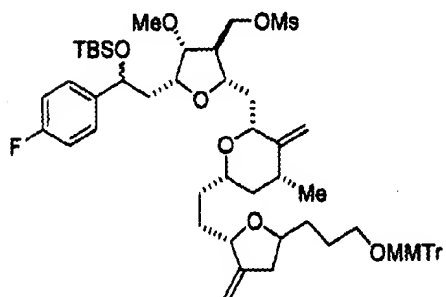
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

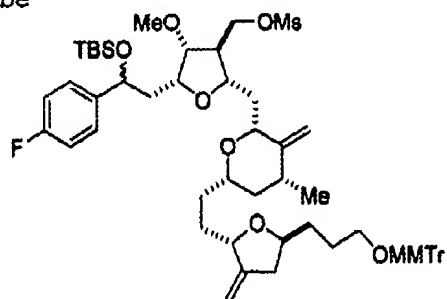
Page 29 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 93,
Line 50: the drawing labeled "208A and 208B"



should be



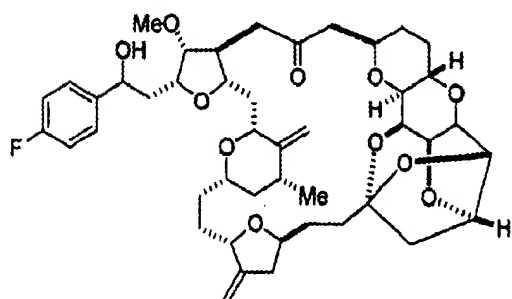
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

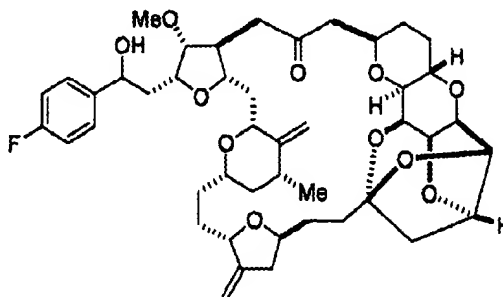
Page 30 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 94,
Line 1: the drawing labeled "B2039, B2043"



should be



Column 94,
Line 43: replace "warn" with -- warm --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

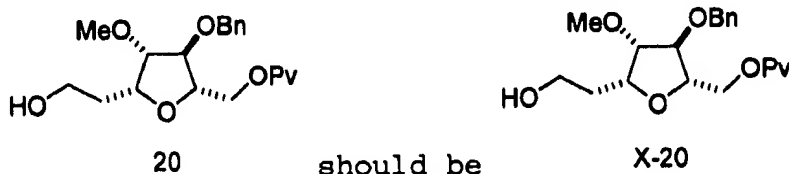
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 31 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 94,

Line 60: the drawing labeled "20" should be -- X-20 -- as follows:

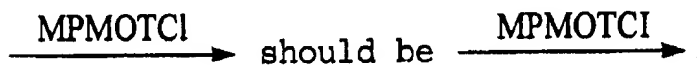


Column 96,

Lines 4-5: replace "NaBO₃·4 H₂O" with -- NaBO₃ · 4H₂O --.

Column 96,

Line 35: the reagent



Column 96,

Line 44: replace "BF₃ · OEt₂" with -- NaBO₃ · OEt₂ --.

Column 97,

Line 59: replace "NaBO₃ · 4 H₂O" with -- NaBO₃ · 4H₂O --.

Column 98,

Line 58: replace "BF₃ · OEt₂" with -- BF₃ · OEt₂ --.

Column 99,

Line 50: replace "Na₂S₂O₅ · 5H₂O" with -- Na₂S₂O₅ · 5H₂O --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

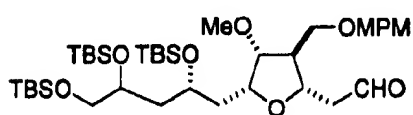
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 32 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

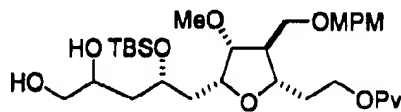
Column 101,

Line 1: replace the drawing labeled "38" with the drawing labeled -- 35a, 35b -- as follows:



38

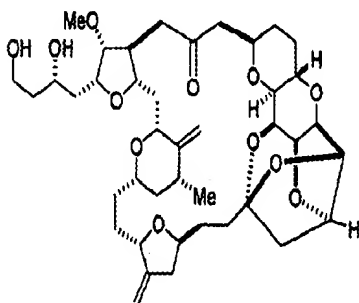
should be



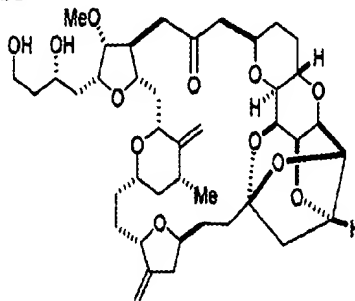
35a, 35b

Column 103,

Line 1: the drawing labeled "B2091"



should be



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

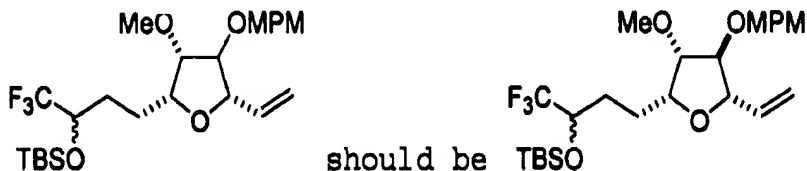
PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 33 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

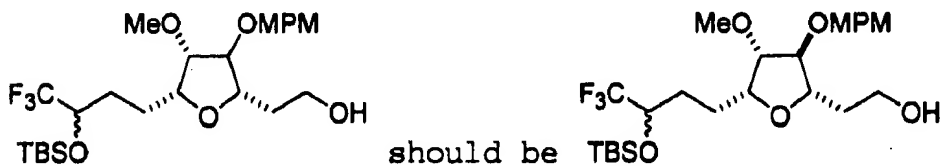
Column 103,
Line 64: replace "NaBO₃ . 4 H₂O" with -- NaBO₃ • 4H₂O --.

Column 106,
Line 60: the drawing labeled "309"



Column 107,
Line 14: replace "NaBO₃ . 4 H₂O" with -- NaBO₃ • 4H₂O --.

Column 107,
Line 20: the drawing labeled "310"



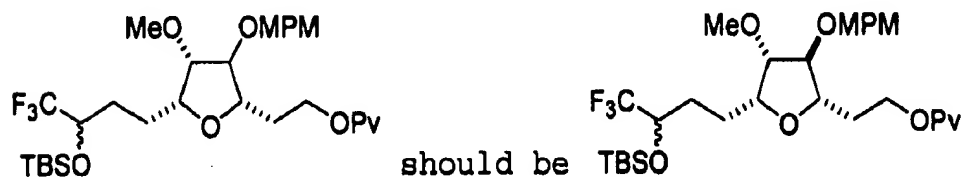
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

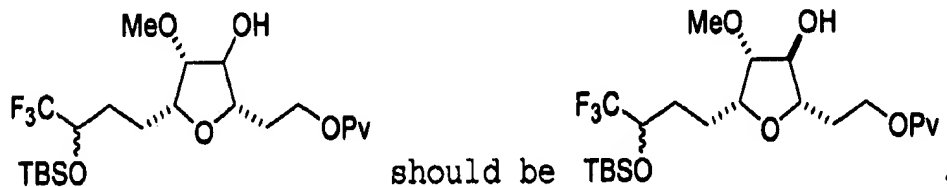
Page 34 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 107,
Line 50: the drawing labeled "311"



Column 108,
Line 12: the drawing labeled "312"



Column 109,
Line 23: replace " $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$ " with -- $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 35 of 41

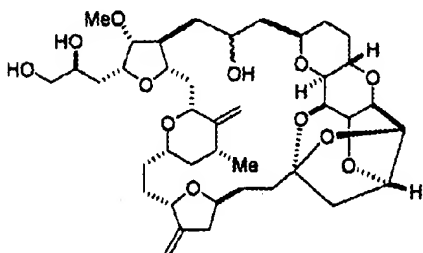
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 110,
Line 5: the reagent

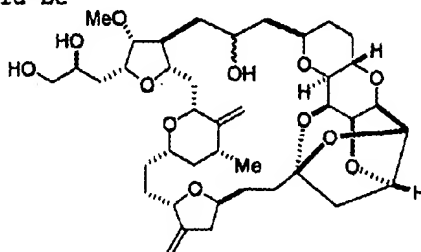
$\xrightarrow{\text{MPMOTCl}}$ should be $\xrightarrow{\text{MPMOTCl}}$.

Column 110,
Line 26: replace " $\text{BF}_3 \cdot \text{OEt}_2$ " with -- $\text{BF}_3 \cdot \text{OEt}_2$ --.

Column 111,
Line 31: the drawing labeled "B1897" should be labeled -- B1896, B1897 --.
Additionally, the drawing labeled "B1897"



should be



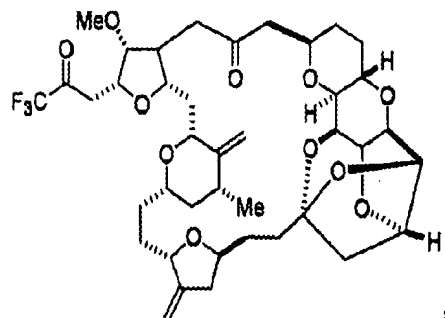
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

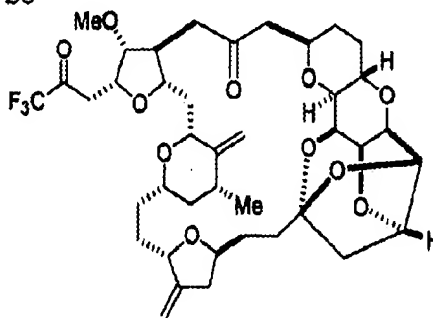
Page 36 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 112,
Line 31: the drawing labeled "B1942"



should be



Column 113,
Line 1: replace "A mixture of B1897" with -- A mixture of B1896/B1897 --.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 37 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

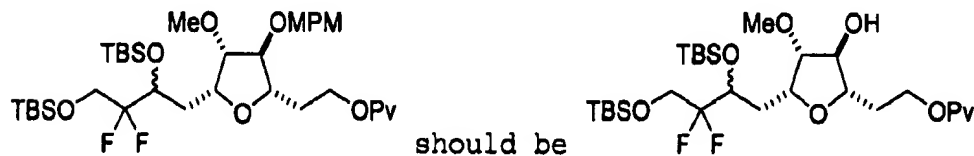
Column 113,
Line 29: replace "finish" with -- furnish --.

Column 113,
Line 30: replace " $C_{40}H_{55}F_3O_{11} + H$ " with -- $C_{40}H_{53}F_3O_{11} + H$ --.

Column 116,
Line 49: replace " $NaBO_3 \cdot 4H_2O$ " with -- $NaBO_3 \cdot 4H_2O$ --.

Column 116,
Lines 51-52: replace " $NaBO_3 \cdot 4H_2O$ " with -- $NaBO_3 \cdot 4H_2O$ --.

Column 117,
Line 25: the drawing labeled "408"



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 38 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

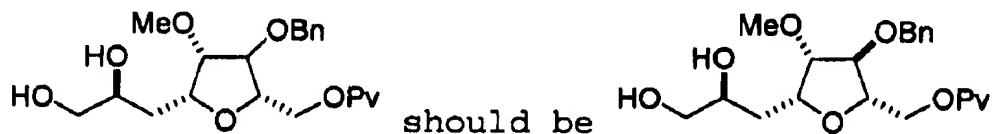
Column 117,
Line 56: replace "TMF" with -- THF --.

Column 118,
Line 21: replace "NaBO₃ . 4H₂O" with -- NaBO₃ • 4H₂O --.

Column 119,
Line 26: replace "BF₃ . OEt₂" with -- BF₃ • OEt₂ --.

Column 119,
Lines 42-43: replace
"412B (46 mg, 36% for 2 steps) as a ~9:1 mixture of C34 isomers"
with
-- 412B:412A (46 mg, 36% for 2 steps) as a ~9:1 mixture of C34 isomers --.

Column 120,
Line 10: the label in the drawing labeled "10" should be -- 10a or 10B --. Additionally,
the drawing labeled "10"



UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

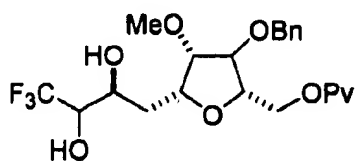
Page 39 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

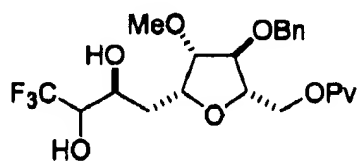
Column 120,
Line 26: replace "diol 10" with -- diol 10a or 10b --.

Column 120,
Line 36: replace "TMF" with -- THF --.

Column 121,
Line 9: the unlabeled drawing should be labeled -- 501 --. Additionally, the unlabeled drawing



should be



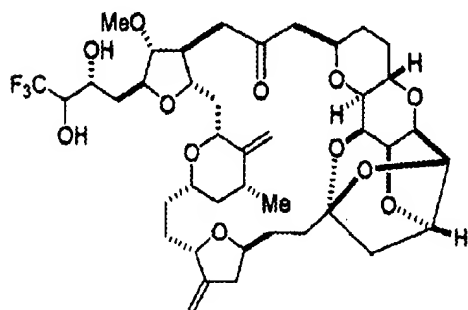
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

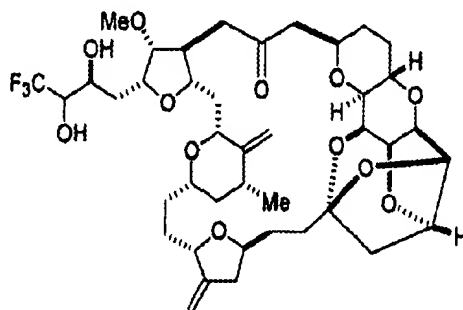
Page 40 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 122,
Line 9: the drawing labeled "B1963"



should be



Column 127,
Line 39: replace "fall range" with -- full range --.

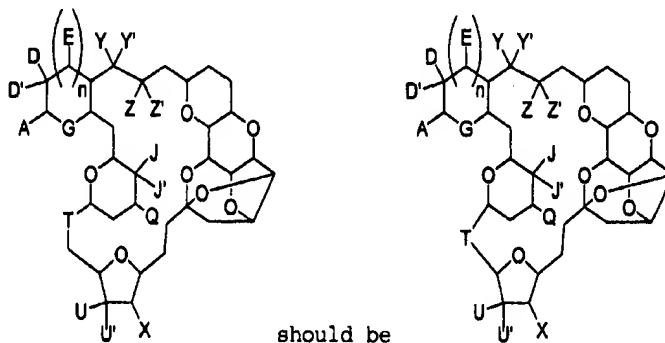
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,214,865 B1
APPLICATION NO. : 09/334488
DATED : April 10, 2001
INVENTOR(S) : Littlefield et al.

Page 41 of 41

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 129, claim 1
Line 13: the drawing



Column 130, claim 7
Line 58: replace "7. The compound of claim 6" with
-- 7. The compound of claim 5 --.

Column 131, claim 16
Line 19: replace "16. The compound of claim 6" with
-- 16. The compound of claim 5 --.

Signed and Sealed this

Twenty-second Day of January, 2008

JON W. DUDAS
Director of the United States Patent and Trademark Office

EXHIBIT 2

Patent Assignment Abstract of Title

NOTE: Results display only for issued patents and published applications. For pending or abandoned applications please consult USPTO staff.

Total Assignments: 2

Patent #: 6214865

Issue Dt: 04/10/2001

Application #: 09334488

Filing Dt: 06/16/1999

Inventors: BRUCE A. LITTLEFIELD, MONICA H. PALME, BORIS M. SELETSKY, MURRAY J. TOWLE, MELVIN J. YU et al

Title: MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATION

Assignment: 1

Reel/Frame: 010118 / 0278

Recorded: 07/26/1999

Pages: 5

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignors: LITTLEFIELD, BRUCE A.

Exec Dt: 07/07/1999

PALME, MONICA H.

Exec Dt: 07/09/1999

SELETSKY, BORIS M.

Exec Dt: 07/07/1999

TOWLE, MURRAY J.

Exec Dt: 07/07/1999

YU, MELVIN J.

Exec Dt: 07/07/1999

ZHENG, WANJUN

Exec Dt: 07/07/1999

Assignee: EISAI CO., LTD.

KOISHIKAWA, BUNKYO-KU 4-6-10

TOKYO, JAPAN

Correspondent: CLARK & ELBING LLP

PAUL T. CLARK

176 FEDERAL STREET

BOSTON, MASSACHUSETTS 02110

Assignment: 2

Reel/Frame: 020352 / 0458

Recorded: 01/11/2008

Pages: 6

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: EISAI CO., LTD.

Exec Dt: 05/11/2007

Assignee: EISAI R&D MANAGEMENT CO., LTD.

6-10, KOISHIKAWA 4-CHOME, BUNKYO-KU

TOKYO, JAPAN 112-8088

Correspondent: SUSAN M. MICHAUD

CLARK & ELBING LLP

101 FEDERAL STREET

BOSTON, MA 02110

Search Results as of: 07/14/2010 04:07 PM

If you have any comments or questions concerning the data displayed, contact PRD / Assignments at 571-272-3350.
Web interface last modified: October 18, 2008 v.2.0.1

MAP
7-26-99
Substitute Form 1

07-28-1999

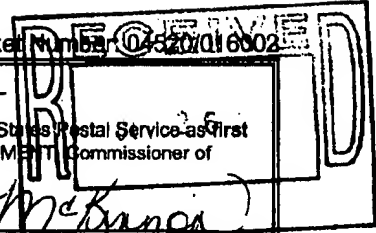


101101745

Certificate of Mailing Date of Deposition

Attorney Docket Number 04520/016502

7/22/99



I hereby certify under 37 CFR 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to BOX ASSIGNMENT, Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Nicky McKinnon

Printed Name

Signature

RECORDATION FORM COVER SHEET
PATENTS ONLY

Commissioner of Patents & Trademarks: Please record the attached document.

1. Names of all conveying parties: 1.) Bruce A. Littlefield 2.) Monica H. Palme 3.) Boris M. Seletsky 4.) Murray J. Towle 5.) Melvin J. Yu 6.) Wanjun Zheng Additional names attached: NO	2. Names and addresses of all receiving parties: Eisai Co., Ltd. Kolshikawa, Bunkyo-Ku 4-6-10 Tokyo, Japan Additional names/addresses attached: NO
3. Nature of conveyance: <input checked="" type="checkbox"/> Assignment <input type="checkbox"/> Merger <input type="checkbox"/> Security Agreement <input type="checkbox"/> Change of Name <input type="checkbox"/> Other: _____ Execution Date: July 7, 1999 and July 9, 1999	
4. Application numbers or patent numbers: A. Patent Application Numbers: 09/334,488	B. Patent Numbers:
5. Name and address of party to whom correspondence concerning document should be mailed: Paul T. Clark, Registration No. 30,162 Clark & Elbing LLP 176 Federal Street Boston, MA 02110	6. Total number of applications/patents involved: 1 7. Total fee (37 CFR 3.41): \$40.00 <input checked="" type="checkbox"/> Fee enclosed <input type="checkbox"/> Authorized to charge deposit account 8. Deposit account number: 03-2095. If the fee above is being charged to deposit account, a duplicate copy of this cover sheet is attached. Please apply any additional charges, or any credits, to Deposit Account No. 03-2095.

DO NOT USE THIS SPACE

07/27/1999 DMBUYEN 00000446 09334488

01 FC:581

40.00 DP

9. Statement and signature: To the best of my knowledge and belief, the foregoing information is true and correct and the attached is the original document.

Susan M. Michaud
Name of person signing

Susan M. Michaud
Signature Reg. No. 42,885

July 22, 1999
Date

ASSIGNMENT

For valuable consideration, we,

Full Name of Assignor	City	State (and Country if not USA)
Bruce A. Littlefield	Andover	MA
Monica H. Palme	San Jose	CA
Boris M. Seletsky	Andover	MA
Murray J. Towle	Auburn	NH
Melvin J. Yu	Andover	MA
Wanjun Zheng	Londonderry	NH

hereby assign to

Full Name of Assignee	State of Incorporation	Business Address
Eisai Co., Ltd.	Japan	Koishikawa, Bunkyo-Ku 4-6-10 Tokyo, JAPAN

and to its successors and assigns (collectively hereinafter called "the Assignee"), the entire right, title and interest throughout the world in the inventions and improvements which are subject of one or more applications for United States Patent signed by us, identified as:

Title of Application	Filing Date	Serial Number
Macrocyclic Analogs and Methods of Their Use and Preparation	June 16, 1999	09/334,488

and we authorize and request the attorneys appointed in said application to hereafter complete this assignment by inserting above the filing date and serial number of said application when known; this assignment includes said application, any and all United States and foreign patents, utility models, and design registrations granted for any of said inventions or improvements, and the right to claim priority based on the filing date of said application under the International Convention for the Protection of Industrial Property, the Patent Cooperation Treaty, the European Patent Convention, and all other treaties of like purposes; and we authorize the Assignee to apply in all countries in our names or in its own name for patents, utility models, design registrations and like rights of exclusion, and for inventors' certificates for said inventions and improvements; and we agree for ourselves and our respective heirs, legal representatives and assigns, without further compensation, to perform such lawful acts and to sign such further applications, assignments, Preliminary Statements and other lawful documents as the Assignee may reasonably request to effectuate fully this assignment.

IN WITNESS WHEREOF, I hereto set my hand and seal at Arlington, Massachusetts, U.S.A.
this 7th day of July, 1999

Bruce A. Littlefield L.S.
Bruce A. Littlefield

STATE OF Massachusetts :ss.
COUNTY OF Essex :

Before me this 7th day of July, 1999, personally appeared Bruce A. Littlefield known to me to be the person whose name is subscribed to the foregoing Assignment, and acknowledged that he/she executed the same as his/her free act and deed for the purposes therein contained.

Michael P. Ryan
Notary Public

My Commission Expires: February 10, 2000

[Notary's Seal Here]

IN WITNESS WHEREOF, I hereto set my hand and seal at Mountain View, CA
this 9th day of July, 1999

Monica H. Palme L.S.
Monica H. Palme

STATE OF California :ss.
COUNTY OF Santa Clara :

Before me this 9th day of July, 1999, personally appeared Monica H. Palme known to me to be the person whose name is subscribed to the foregoing Assignment, and acknowledged that he/she executed the same as his/her free act and deed for the purposes therein contained.

Sarah V. Chang
Notary Public

My Commission Expires:
July 24, 2002



[Notary's Seal Here]

IN WITNESS WHEREOF, I hereto set my hand and seal at Andover, Massachusetts, U.S.A.
this 7th day of July, 1999

B. Seletsky L.S.

Boris M. Seletsky

STATE OF Massachusetts:

COUNTY OF Essex: ss.

Before me this 7th day of July, 1999, personally appeared Boris M. Seletsky known to me to be the person whose name is subscribed to the foregoing Assignment, and acknowledged that he/she executed the same as his/her free act and deed for the purposes therein contained.

Michael P. Liguori
Notary Public

My Commission Expires: February 10, 2000

[Notary's Seal Here]

IN WITNESS WHEREOF, I hereto set my hand and seal at Andover, Massachusetts, U.S.A.
this 7th day of July, 1999

Murray J. Towle L.S.
Murray J. Towle

STATE OF Massachusetts:

COUNTY OF Essex: ss.

Before me this 7th day of July, 1999, personally appeared Murray J. Towle known to me to be the person whose name is subscribed to the foregoing Assignment, and acknowledged that he/she executed the same as his/her free act and deed for the purposes therein contained.

Michael P. Liguori
Notary Public

My Commission Expires: February 10, 2000

[Notary's Seal Here]

IN WITNESS WHEREOF, I hereto set my hand and seal at Andover, Massachusetts, U.S.A.
this 7th day of July, 1999

Melvin J. Yu L.S.
Melvin J. Yu

STATE OF Massachusetts:

COUNTY OF Essex: :ss.

Before me this 7th day of July, 1999, personally appeared Melvin J. Yu
known to me to be the person whose name is subscribed to the foregoing Assignment, and acknowledged
that he/she executed the same as his/her free act and deed for the purposes therein contained.

Richard P. Lipman
Notary Public

My Commission Expires: February 10, 2000

[Notary's Seal Here]

IN WITNESS WHEREOF, I hereto set my hand and seal at Andover, Massachusetts, U.S.A.
this 7th day of July, 1999

Wanjun Zheng L.S.
Wanjun Zheng

STATE OF Massachusetts:

COUNTY OF Essex: :ss.

Before me this 7th day of July, 1999, personally appeared Wanjun Zheng
known to me to be the person whose name is subscribed to the foregoing Assignment, and acknowledged
that he/she executed the same as his/her free act and deed for the purposes therein contained.

Richard P. Lipman
Notary Public

My Commission Expires: February 10, 2000

[Notary's Seal Here]

PATENT ASSIGNMENT

Electronic Version v1.1
 Stylesheet Version v1.1

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT
CONVEYING PARTY DATA	
Name	Execution Date
Eisai Co., Ltd.	05/11/2007
RECEIVING PARTY DATA	
Name:	Eisai R&D Management Co., Ltd.
Street Address:	6-10, Koishikawa 4-chome, Bunkyo-ku
City:	Tokyo
State/Country:	JAPAN
Postal Code:	112-8088
PROPERTY NUMBERS Total: 5	
Property Type	Number
Application Number:	60089682
Application Number:	09334488
Application Number:	09677485
Application Number:	09843617
PCT Number:	US9913677
CORRESPONDENCE DATA	
Fax Number:	(617)428-7045
<i>Correspondence will be sent via US Mail when the fax attempt is unsuccessful.</i>	
Phone:	6174280200
Email:	patentadministrator@clarkelbing.com
Correspondent Name:	Susan M. Michaud
Address Line 1:	Clark & Elbing LLP
Address Line 2:	101 Federal Street
Address Line 4:	Boston, MASSACHUSETTS 02110
ATTORNEY DOCKET NUMBER:	04520/016003
NAME OF SUBMITTER:	Susan M. Michaud

PATENT

500437438

REEL: 020352 FRAME: 0458

CH \$200.00 60089682

Total Attachments: 4

source=04520.001001 Assignment of All U.S. cases#page1.tif

source=04520.001001 Assignment of All U.S. cases#page2.tif

source=04520.001001 Assignment of All U.S. cases#page3.tif

source=04520.001001 Assignment of All U.S. cases#page4.tif

PATENT

REEL: 020352 FRAME: 0459

ASSIGNMENT

For valuable consideration, we,

Full Name of Assignor	Business Address
Eisai Co., Ltd.	6-10, Koishikawa 4-chome, Bunkyo-ku Tokyo 112-8088, Japan


hereby assign to

Full Name of Assignee	Business Address
Eisai R&D Management Co., Ltd.	6-10, Koishikawa 4-chome, Bunkyo-ku Tokyo 112-8088, Japan

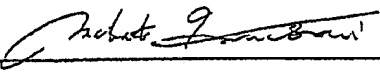
and to its successors and assigns (collectively hereinafter called "the Assignee"), our entire right, title, and interest throughout the world in the inventions and improvements which are subject of one or more applications for United States Patent, listed on the attached Appendix A.

This assignment includes said applications, any and all United States and foreign patents, utility models, and design registrations granted for any of said inventions or improvements, and the right to claim priority based on the filing date of said application under the International Convention for the Protection of Industrial Property, the Patent Cooperation Treaty, the European Patent Convention, and all other treaties of like purposes; and we authorize the Assignee to apply in all countries in our names or in its own name for patents, utility models, design registrations, and like rights of exclusion, and for inventors' certificates for said inventions and improvements; and we agree for ourselves and our respective heirs, legal representatives and assigns, without further compensation, to perform such lawful acts and to sign such further applications, assignments, Preliminary Statements, and other lawful documents as the Assignee may reasonably request to effectuate fully this assignment. This assignment also includes the right to sue for past acts of infringement, whether based on any patents listed herein, patents issuing from applications listed herein, or provisional rights from any patent applications listed herein.

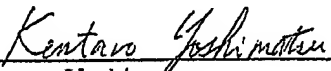
ASSIGNOR:

Signature:  Date: May 11. 2007
Nobuo Deguchi
Senior Vice President, Intellectual Property
Eisai Co., Ltd.

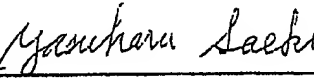
Witness:

Signature:  Date: May 11. 2007
Printed name: MAKOTO KAMOKARI

ASSIGNEE:

Signature:  Date: May 18. 2007
Kentaro Yoshimatsu
President
Eisai R&D Management Co., Ltd.

Witness:

Signature:  Date: May 18. 2007
Printed name: Yasuharu Saeki

Appendix A		
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Appendix A		
Application Number	Filing Date	U.S. Patent Number
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EXHIBIT 3

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use HALAVEN™ safely and effectively. See full prescribing information for HALAVEN.

HALAVEN™ (eribulin mesylate) Injection

For intravenous administration only.

Eisai Inc.

Initial US Approval: 2010

INDICATIONS AND USAGE

- HALAVEN is a microtubule inhibitor indicated for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting (1).

DOSAGE AND ADMINISTRATION

- Administer 1.4 mg/m² intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle (2.1).
- Reduce dose in patients with hepatic impairment and moderate renal impairment (2.1).
- Do not mix with other drugs or administer with dextrose-containing solutions (2.3).

DOSAGE FORMS AND STRENGTHS

Intravenous administration.

Eribulin mesylate injection, 1 mg per 2 mL (0.5 mg per mL) (3).

CONTRAINDICATIONS

None.

WARNINGS AND PRECAUTIONS

- Neutropenia: Monitor peripheral blood cell counts and adjust dose as appropriate (2.2, 5.1, 6).
- Peripheral Neuropathy: Monitor for signs of neuropathy. Manage with dose delay and adjustment (2.2, 5.2, 6).
- Use in Pregnancy: Fetal harm can occur when administered to a pregnant woman (5.3) (8.1).
- QT Prolongation: Monitor for prolonged QT intervals in patients with congestive heart failure, bradyarrhythmias, drugs known to prolong the QT interval, and electrolyte abnormalities. Avoid in patients with congenital long QT syndrome (5.4).

ADVERSE REACTIONS

The most common adverse reactions (incidence ≥25%) were neutropenia, anemia, asthenia/fatigue, alopecia, peripheral neuropathy, nausea, and constipation (6).

To report SUSPECTED ADVERSE REACTIONS, contact Eisai Inc. at (1-877-873-4724) or contact FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

USE IN SPECIFIC POPULATIONS

- Nursing Mothers: Discontinue drug or nursing, taking into consideration the importance of the drug to the mother (8.3).
- Hepatic Impairment: A lower starting dose is recommended for patients with mild (Child-Pugh A) and moderate (Child-Pugh B) hepatic impairment. Patients with severe hepatic impairment (Child-Pugh C) were not studied (8.6).
- Renal Impairment: A lower starting dose is recommended for patients with moderate (CrCl 30-50 mL/min) renal impairment. Patients with severe (CrCl < 30 mL/min) renal impairment were not studied (8.7).

See 17 for PATIENT COUNSELING INFORMATION and FDA-Approved Patient Labeling.

Revised: 11/2010

FULL PRESCRIBING INFORMATION: CONTENTS*

FULL PRESCRIBING INFORMATION [FINAL]

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2	DOSAGE AND ADMINISTRATION
2.1	Recommended Dose
2.2	Dose Modification
2.3	Instructions for Preparation and Administration
3	DOSAGE FORMS AND STRENGTHS
4	CONTRAINDICATIONS
5	WARNINGS AND PRECAUTIONS
5.1	Neutropenia
5.2	Peripheral Neuropathy
5.3	Embryo-Fetal Toxicity
5.4	QT Prolongation
6	ADVERSE REACTIONS
7	DRUG INTERACTIONS
7.1	Effects of Other Drugs on HALAVEN
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8	USE IN SPECIFIC POPULATIONS
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8.4	Pediatric Use
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10	OVERDOSAGE
11	DESCRIPTION
12	CLINICAL PHARMACOLOGY
12.1	Mechanism of Action
12.2	Pharmacodynamics
12.3	Pharmacokinetics
13	NONCLINICAL TOXICOLOGY
13.1	Carcinogenesis, mutagenesis, impairment of fertility
14	CLINICAL STUDIES
16	HOW SUPPLIED / STORAGE AND HANDLING
17	PATIENT COUNSELING INFORMATION

* Sections or subsections omitted from the full prescribing information are not listed.

1 INDICATIONS AND USAGE

HALAVEN is indicated for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting.

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Dose

The recommended dose of HALAVEN is 1.4 mg/m² administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle.

The recommended dose of HALAVEN in patients with mild hepatic impairment (Child-Pugh A) is 1.1 mg/m² administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle. [see *Use in Specific Populations* (8.6)]

The recommended dose of HALAVEN in patients with moderate hepatic impairment (Child-Pugh B) is 0.7 mg/m² administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle. [see *Use in Specific Populations* (8.6)]

The recommended dose of HALAVEN in patients with moderate renal impairment (creatinine clearance of 30-50 mL/min) is 1.1 mg/m² administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle. [see *Use in Specific Populations* (8.7)]

2.2 Dose Modification

Assess for peripheral neuropathy and obtain complete blood cell counts prior to each dose.

Recommended dose delays

- Do not administer HALAVEN on Day 1 or Day 8 for any of the following:
 - ANC < 1,000/mm³
 - Platelets < 75,000/mm³
 - Grade 3 or 4 non-hematological toxicities.
- The Day 8 dose may be delayed for a maximum of 1 week.
 - If toxicities do not resolve or improve to ≤ Grade 2 severity by Day 15, omit the dose.
 - If toxicities resolve or improve to ≤ Grade 2 severity by Day 15, administer HALAVEN at a reduced dose and initiate the next cycle no sooner than 2 weeks later.

Recommended dose reductions

- If a dose has been delayed for toxicity and toxicities have recovered to Grade 2 severity or less, resume HALAVEN at a reduced dose as set out in Table 1.
- Do not re-escalate HALAVEN dose after it has been reduced.

Table 1 Recommended Dose Reductions

Event Description	Recommended HALAVEN Dose
Permanently reduce the 1.4 mg/m² HALAVEN dose for any of the following:	
ANC <500/mm ³ for >7 days	1.1 mg/m ²
ANC <1,000 /mm ³ with fever or infection	
Platelets <25,000/mm ³	
Platelets <50,000/mm ³ requiring transfusion	
Non-hematologic Grade 3 or 4 toxicities	
Omission or delay of Day 8 HALAVEN dose in previous cycle for toxicity	
Occurrence of any event requiring permanent dose reduction while receiving 1.1 mg/m²	0.7 mg/m ²
Occurrence of any event requiring permanent dose reduction while receiving 0.7 mg/m²	Discontinue HALAVEN
ANC = absolute neutrophil count. Toxicities graded in accordance with National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.	

2.3 Instructions for Preparation and Administration

Aseptically withdraw the required amount of HALAVEN from the single-use vial and administer undiluted or diluted in 100 mL of 0.9% Sodium Chloride Injection, USP.

Do not dilute in or administer through an intravenous line containing solutions with dextrose. Do not administer in the same intravenous line concurrent with the other medicinal products.

Store undiluted HALAVEN in the syringe for up to 4 hours at room temperature or for up to 24 hours under refrigeration (40°F or/ 4°C). Store diluted solutions of HALAVEN for up to 4 hours at room temperature or up to 24 hours under refrigeration.

Discard unused portions of the vial.

3 DOSAGE FORMS AND STRENGTHS

HALAVEN (eribulin mesylate) Injection, 1 mg/2 mL (0.5 mg/mL).

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Neutropenia

Severe neutropenia ($\text{ANC} < 500/\text{mm}^3$) lasting more than one week occurred in 12% (62/503) of patients in Study 1, leading to discontinuation in $<1\%$ of patients [see *Adverse Reactions* (6)]. Patients with alanine aminotransferase or aspartate aminotransferase $> 3 \times \text{ULN}$ (upper limit of normal) experienced a higher incidence of Grade 4 neutropenia and febrile neutropenia than patients with normal aminotransferase levels. Patients with bilirubin $> 1.5 \times \text{ULN}$ also had a higher incidence of Grade 4 neutropenia and febrile neutropenia.

Monitor complete blood counts prior to each dose; increase the frequency of monitoring in patients who develop Grade 3 or 4 cytopenias. Delay administration of HALAVEN and reduce subsequent doses in patients who experience febrile neutropenia or Grade 4 neutropenia lasting longer than 7 days [see *Dosage and Administration* (2.2)]. Clinical studies of HALAVEN did not include patients with baseline neutrophil counts below $1,500/\text{mm}^3$.

5.2 Peripheral Neuropathy

Grade 3 peripheral neuropathy occurred in 8% (40/503) of patients, and Grade 4 in 0.4% (2/503) of patients in Study 1. Peripheral neuropathy was the most common toxicity leading to discontinuation of HALAVEN (5% of patients; 24/503). Neuropathy lasting more than one year occurred in 5% (26/503) of patients. Twenty-two percent (109/503) of patients developed a new or worsening neuropathy that had not recovered within a median follow-up duration of 269 days (range 25-662 days). Monitor patients closely for signs of peripheral motor and sensory neuropathy. Withhold HALAVEN in patients who experience Grade 3 or 4 peripheral neuropathy until resolution to Grade 2 or less [see *Dosage and Administration* (2.2)].

5.3 Embryo-Fetal Toxicity

There are no adequate and well-controlled studies of HALAVEN in pregnant women. HALAVEN is a microtubule inhibitor; therefore, it is expected to cause fetal harm when administered to a pregnant woman. Embryo-fetal toxicity and teratogenicity occurred in rats that received eribulin mesylate at approximately half of the recommended human dose based on body surface area. If this drug is used during pregnancy, or if a patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus [see *Use in Specific Populations* (8.1)].

5.4 QT Prolongation

In an uncontrolled open-label ECG study in 26 patients, QT prolongation was observed on Day 8, independent of eribulin concentration, with no QT prolongation observed on Day 1. ECG monitoring is recommended if therapy is initiated in patients with congestive heart failure, bradyarrhythmias, drugs known to prolong the QT interval, including Class Ia and III antiarrhythmics, and electrolyte abnormalities. Correct hypokalemia or hypomagnesemia prior to initiating HALAVEN and monitor these electrolytes periodically during therapy. Avoid HALAVEN in patients with congenital long QT syndrome.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in detail in other sections of the labeling:

- Neutropenia [see *Warnings and Precautions* (5.1)]
- Peripheral neuropathy [see *Warnings and Precautions* (5.2)]
- QT interval prolongation [see *Warnings and Precautions* (5.4)].

The most common adverse reactions ($\geq 25\%$) reported in patients receiving HALAVEN were neutropenia, anemia, asthenia/fatigue, alopecia, peripheral neuropathy, nausea, and constipation.

The most common serious adverse reactions reported in patients receiving HALAVEN were febrile neutropenia (4%) and neutropenia (2%). The most common adverse reaction resulting in discontinuation of HALAVEN was peripheral neuropathy (5%).

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in other clinical trials and may not reflect the rates observed in clinical practice.

In clinical trials, HALAVEN has been administered to 1,222 patients with multiple tumor types, including 240 patients exposed to HALAVEN for 6 months or longer. The majority of the 1,222 patients were women (82%) with a median age of 58 years (range: 26 to 91 years). The racial and ethnic distribution was Caucasian (83%), Black (5%), Asian (2%), and other (5%).

The adverse reactions described in Table 2 were identified in 750 patients treated in Study 1 [*see Clinical Studies (14)*]. In Study 1, patients were randomized (2:1) to receive either HALAVEN (1.4 mg/m² on Days 1 and 8 of a 21-day cycle) or single agent treatment chosen by their physician (control group). A total of 503 patients received HALAVEN, and 247 patients in the control group received therapy consisting of chemotherapy [total 97% (anthracyclines 10%, capecitabine 18%, gemcitabine 19%, taxanes 15%, vinorelbine 25%, other chemotherapies 10%)] or hormonal therapy (3%). The median duration of exposure was 118 days for patients receiving HALAVEN and 63 days for patients receiving control therapy. Table 2 reports the most common adverse reactions occurring in at least 10% of patients in either group.

Table 2 Adverse Reactions with a Per-Patient Incidence of at Least 10% in Study 1

MedDRA ver 10.0	HALAVEN n=503		Control Group n=247	
	All Grades	≥ Grade 3	All Grades	≥ Grade 3
Blood and Lymphatic System Disorders^a				
Neutropenia	82%	57%	53%	23%
Anemia	58%	2%	55%	4%
Nervous system disorders				
Peripheral neuropathy ^b	35%	8%	16%	2%
Headache	19%	<1%	12%	<1%
General disorders and administrative site conditions				
Asthenia/Fatigue	54%	10%	40%	11%
Mucosal inflammation	9%	1%	10%	2%
Pyrexia	21%	<1%	13%	<1%
Gastrointestinal disorders				
Constipation	25%	1%	21%	1%
Diarrhea	18%	0	18%	0
Nausea	35%	1%	28%	3%
Vomiting	18%	1%	18%	1%
Musculoskeletal and connective tissue disorders				
Arthralgia/Myalgia	22%	<1%	12%	1%
Back pain	16%	1%	7%	2%
Bone pain	12%	2%	9%	2%
Pain in extremity	11%	1%	10%	1%
Investigations				
Weight decreased	21%	1%	14%	<1%
Metabolism and nutrition disorders				
Anorexia	20%	1%	13%	1%
Respiratory, thoracic, and mediastinal disorders				
Cough	14%	0	9%	0
Dyspnea	16%	4%	13%	4%
Skin and subcutaneous tissue disorders				
Alopecia	45%	NA ^c	10%	NA ^c
Infections and Infestations				
Urinary Tract Infection	10%	1%	5%	0

^a based upon laboratory data.

^b includes neuropathy peripheral, neuropathy, peripheral motor neuropathy, polyneuropathy, peripheral sensory neuropathy, and paraesthesia.

^c not applicable; (grading system does not specify > Grade 2 for alopecia).

Cytopenias: Grade 3 neutropenia occurred in 28% (143/503) of patients who received HALAVEN in Study 1, and 29% (144/503) of patients experienced Grade 4 neutropenia. Febrile neutropenia occurred in 5% (23/503) of patients; two patients (0.4%) died from complications of febrile neutropenia. Dose reduction due to neutropenia was required in 12% (62/503) of patients and discontinuation was required in <1% of patients. The mean time to nadir was 13 days and the mean time to recovery from severe neutropenia (<500/mm³) was 8 days. Grade 3 or greater thrombocytopenia occurred in 1% (7/503) of patients. G-CSF (granulocyte colony-stimulating factor) or GM-CSF (granulocyte-macrophage colony-stimulating factor) was used in 19% of patients who received HALAVEN.

Peripheral Neuropathy: In Study 1, 17 % of enrolled patients had Grade 1 peripheral neuropathy and 3% of patients had Grade 2 peripheral neuropathy at baseline. Dose reduction due to peripheral neuropathy was required by 3% (14/503) of patients who received HALAVEN. Four percent (20/503) of patients experienced peripheral motor neuropathy of any grade and 2% (8/503) of patients developed Grade 3 peripheral motor neuropathy.

Liver Function Test Abnormalities: Among patients with Grade 0 or 1 ALT levels at baseline, 18% of HALAVEN-treated patients experienced Grade 2 or greater ALT elevation. One HALAVEN-treated patient without documented liver metastases had concomitant Grade 2 elevations in bilirubin and ALT; these abnormalities resolved and did not recur with re-exposure to HALAVEN.

Less Common Adverse Reactions: The following additional adverse reactions were reported in $\geq 5\%$ to $< 10\%$ of the HALAVEN-treated group:

- **Eye Disorders:** increased lacrimation
- **Gastrointestinal Disorders:** dyspepsia, abdominal pain, stomatitis, dry mouth
- **General Disorders and Administration Site Conditions:** peripheral edema
- **Infections and Infestations:** upper respiratory tract infection
- **Metabolism and Nutrition Disorders:** hypokalemia
- **Musculoskeletal and Connective Tissue Disorders:** muscle spasms, muscular weakness
- **Nervous System Disorders:** dysgeusia, dizziness
- **Psychiatric Disorders:** insomnia, depression
- **Skin and Subcutaneous Tissue Disorders:** rash

7 DRUG INTERACTIONS

7.1 Effects of Other Drugs on HALAVEN

No drug-drug interactions are expected with CYP3A4 inhibitors or P-gp inhibitors. The effect of ketoconazole, a strong inhibitor of cytochrome P450 3A4 (CYP3A4) and a P-gp inhibitor, on the pharmacokinetics (PK) of eribulin was studied in an open-label, two-treatment, two-sequence, two-way crossover trial in 12 patients with advanced solid tumors. The mean dose-normalized AUC values were similar when eribulin was administered with or without ketoconazole (ratio of the mean AUC: 0.97; 90% CI: 0.83, 1.12).

7.2 Effect of HALAVEN on Other Drugs

Eribulin does not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 enzymes or induce CYP1A2, CYP2C9, CYP2C19 or CYP3A4 enzymes at relevant clinical concentrations. Eribulin is not expected to alter the plasma concentrations of drugs that are substrates of these enzymes [see *Clinical Pharmacology* (12.3)].

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy Category D [see *Warnings and Precautions* (5.3)]

There are no adequate and well-controlled studies with HALAVEN in pregnant women. HALAVEN is a microtubule inhibitor, therefore, it is expected to cause fetal harm when administered to a pregnant woman. Embryo-fetal toxicity and teratogenicity occurred in rats that received eribulin mesylate at approximately half of the recommended human dose based on body surface area. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

In a developmental toxicity study, pregnant rats received intravenous infusion of eribulin mesylate during organogenesis (Gestation Days 8, 10, and 12) at doses approximately 0.04, 0.13, 0.43 and 0.64 times the recommended human dose, based on body surface area (mg/m^2). Increased abortion and severe external or soft tissue malformations were observed in offspring at doses 0.64 times the recommended human dose based on body surface area (mg/m^2), including the absence of a lower jaw, tongue, stomach and spleen. Increased embryo-fetal death/resorption,

reduced fetal weights, and minor skeletal anomalies consistent with developmental delay were also reported at or above doses of 0.43 times the recommended human dose.

Maternal toxicity of eribulin mesylate was reported in rats at or above doses of 0.43 times the recommended human dose (mg/m²), and included enlarged spleen, reduced maternal weight gain and decreased food consumption.

8.3 Nursing Mothers

It is not known whether HALAVEN is excreted into human milk. No studies in humans or animals were conducted to determine if HALAVEN is excreted into milk. Because many drugs are excreted into human milk and because of the potential for serious adverse reactions in human milk fed infants from HALAVEN, a decision should be made whether to discontinue nursing or to discontinue HALAVEN taking into account the importance of the drug to the mother.

8.4 Pediatric Use

The safety and effectiveness of HALAVEN in pediatric patients below the age of 18 years have not been established.

8.5 Geriatric Use

Study 1 did not include sufficient numbers of subjects aged 65 years and older to determine whether they respond differently from younger subjects. Of the 827 subjects who received the recommended dose and schedule of HALAVEN in clinical studies, 15% (121/827) were 65 and older, and 2% (17/827) patients were 75 and older. No overall differences in safety were observed between these subjects and younger subjects.

8.6 Hepatic Impairment

A study evaluated the PK of eribulin in patients with mild (Child-Pugh A; n=7) and moderate (Child-Pugh B; n=5) hepatic impairment. Compared to patients with normal hepatic function (n=6), eribulin exposure increased 1.8-fold and 2.5-fold in patients with mild and moderate hepatic impairment, respectively. Administration of HALAVEN at a dose of 1.1 mg/m² to patients with mild hepatic impairment and 0.7 mg/m² to patients with moderate hepatic impairment resulted in similar exposure to eribulin as a dose of 1.4 mg/m² to patients with normal hepatic function. A lower starting dose of 1.1 mg/m² is recommended for patients with mild hepatic impairment (Child-Pugh A) and of 0.7 mg/m² is recommended for patients with moderate hepatic impairment (Child-Pugh B). HALAVEN was not studied in patients with severe hepatic impairment (Child-Pugh C). [see *Dosage and Administration* (2.1)]

8.7 Renal Impairment

No formal PK trials were conducted with HALAVEN in patients with renal impairment. Available data suggests that no dose adjustment is necessary for patients with mild renal impairment (CrCl 50-80 mL/min). However, for patients with moderate renal impairment (CrCl 30-50 mL/min), the geometric mean dose-normalized systemic exposure increased 2-fold compared to patients with normal renal function. A lower starting dose of 1.1 mg/m² is recommended for patients with moderate renal impairment. The safety of HALAVEN was not studied in patients with severe renal impairment (CrCl < 30 mL/min). [see *Dosage and Administration* (2.1)]

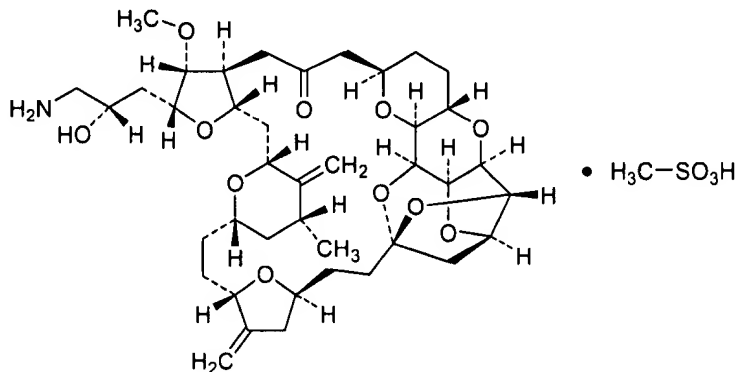
10 OVERDOSAGE

Overdosage of HALAVEN has been reported at approximately 4 times the recommended dose, which resulted in Grade 3 neutropenia lasting seven days and a Grade 3 hypersensitivity reaction lasting one day.

There is no known antidote for HALAVEN overdose.

11 DESCRIPTION

HALAVEN (eribulin mesylate) Injection is a non-taxane microtubule dynamics inhibitor. Eribulin mesylate is a synthetic analogue of halichondrin B, a product isolated from the marine sponge *Halichondria okadai*. The chemical name for eribulin mesylate is 11,15:18,21:24,28-Triepoxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one, 2-[(2*S*)-3-amino-2-hydroxypropyl]hexacosahydro-3-methoxy-26-methyl-20,27-bis(methylene)-, (2*R*,3*R*,3*aS*,7*R*,8*aS*,9*S*,10*aR*,11*S*,12*R*,13*aR*,13*bS*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29*aS*)-, methanesulfonate (salt). It has a molecular weight of 826.0 (729.9 for free base). The empirical formula is $C_{40}H_{59}NO_{11} \cdot CH_4O_3S$. Eribulin mesylate has the following structural formula:



HALAVEN is a clear, colorless, sterile solution for intravenous administration. Each vial contains 1 mg of eribulin mesylate as a 0.5 mg/mL solution in ethanol: water (5:95).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Eribulin inhibits the growth phase of microtubules without affecting the shortening phase and sequesters tubulin into nonproductive aggregates. Eribulin exerts its effects via a tubulin-based antimetabolic mechanism leading to G₂/M cell-cycle block, disruption of mitotic spindles, and, ultimately, apoptotic cell death after prolonged mitotic blockage.

12.2 Pharmacodynamics

Cardiac Electrophysiology

The effect of HALAVEN on the QTc interval was assessed in an open-label, uncontrolled, multicenter, single-arm dedicated QT trial. A total of 26 patients with solid tumors received 1.4 mg/m² of HALAVEN on Days 1 and 8 of a 21-day cycle. A delayed QTc prolongation was observed on Day 8, with no prolongation observed on Day 1. The maximum mean QTcF change from baseline (95% upper confidence interval) was 11.4 (19.5) ms.

12.3 Pharmacokinetics

The pharmacokinetics of eribulin is linear with a mean elimination half-life of approximately 40 hours, a mean volume of distribution of 43 L/m² to 114 L/m² and mean clearance of 1.16 L/hr/m² to 2.42 L/hr/m² over the dose range of 0.25 mg/m² to 4.0 mg/m². The human plasma protein binding of eribulin at concentrations of 100 ng/mL to 1,000 ng/mL ranges from 49% to 65%. Eribulin exposure after multiple dosing is comparable to that following a single dose. No accumulation of eribulin is observed with weekly administration.

Metabolism

Unchanged eribulin was the major circulating species in plasma following administration of ^{14}C -eribulin to patients. Metabolite concentrations represented <0.6% of parent compound, confirming that there are no major human metabolites of eribulin.

Cytochrome P450 3A4 (CYP3A4) negligibly metabolizes eribulin *in vitro*. Eribulin inhibits CYP3A4 activity in human liver microsomes, but it is unlikely that eribulin will substantially increase the plasma levels of CYP3A4 substrates. Eribulin shows no induction potential for CYP1A, CYP2C9, CYP2C19, and CYP3A in primary human hepatocytes. No significant inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP2E1 was detected with eribulin concentrations up to 5 μM in pooled human liver microsomes. *In vitro* drug interaction studies indicate that eribulin does not inhibit drugs that are substrates of these enzymes and it is unlikely that eribulin will affect plasma levels of drugs that are substrates of CYP enzymes. Eribulin is a substrate and a weak inhibitor of the drug efflux transporter P-gp *in vitro*.

Elimination

Eribulin is eliminated primarily in feces unchanged. After administration of ^{14}C -eribulin to patients, approximately 82% of the dose was eliminated in feces and 9% in urine. Unchanged eribulin accounted for approximately 88% and 91% of the dose in feces and urine, respectively.

Effects of Age, Gender, and Race

Based on a population pharmacokinetic analysis with data collected from 340 patients, gender, race, and age do not have a clinically meaningful effect on the PK of eribulin.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, mutagenesis, impairment of fertility

Carcinogenicity studies have not been conducted with eribulin mesylate.

Eribulin mesylate was not mutagenic in *in vitro* bacterial reverse mutation assays (Ames test). Eribulin mesylate was positive in mouse lymphoma mutagenesis assays, and was clastogenic in an *in vivo* rat bone marrow micronucleus assay.

The effects of HALAVEN on human fertility are unknown. Fertility studies have not been conducted with eribulin mesylate in humans or animals. However, nonclinical findings in repeated-dose dog and rat toxicology studies suggest that male fertility may be compromised by treatment with eribulin mesylate. Rats exhibited testicular toxicity (hypocellularity of seminiferous epithelium with hypospermia/aspermia) following dosing with eribulin mesylate at or above 0.43 times the recommended human dose (mg/m^2) given once weekly for 3 weeks, or at or above 0.21 times the recommended human dose (mg/m^2) given once weekly for 3 out of 5 weeks, repeated for 6 cycles. Testicular toxicity was also observed in dogs given 0.64 times the recommended human dose (mg/m^2) weekly for 3 out of 5 weeks, repeated for 6 cycles.

14 CLINICAL STUDIES

Study 1 was an open-label, randomized, multicenter trial of 762 patients with metastatic breast cancer who received at least two chemotherapeutic regimens for the treatment of metastatic disease and experienced disease progression within 6 months of their last chemotherapeutic regimen. Patients were required to receive prior anthracycline- and taxane- based chemotherapy for adjuvant or metastatic disease. Patients were randomized (2:1) to receive HALAVEN ($n=508$) or a single agent therapy selected prior to randomization (control arm, $n=254$). Randomization was stratified by geographic region, HER2/*neu* status, and prior capecitabine exposure. HALAVEN was administered at a dose of $1.4 \text{ mg}/\text{m}^2$ on Days 1 and 8 of a 21-day cycle. HALAVEN-treated patients received a median of 5 cycles (range: 1 to 23 cycles) of therapy.

Control arm therapy consisted of 97% chemotherapy (26% vinorelbine, 18% gemcitabine, 18% capecitabine, 16% taxane, 9% anthracycline, 10% other chemotherapy), and 3% hormonal therapy. The main efficacy outcome was overall survival.

Patient demographic and baseline characteristics were comparable between the treatment arms. The median age was 55 (range: 27 to 85 years) and 92% were White. Sixty-four percent of patients were enrolled in North America/Western Europe/Australia, 25% in Eastern Europe/Russia, and 11% in Latin America/South Africa. Ninety-one percent of patients had a baseline ECOG performance status of 0 or 1. Tumor prognostic characteristics, including estrogen receptor status (positive: 67%, negative: 28%), progesterone receptor status (positive: 49%, negative: 39%), HER2/*neu* receptor status (positive: 16%, negative: 74%), triple negative status (ER-, PR-, HER2/*neu*+: 19%), presence of visceral disease (82%, including 60% liver and 38% lung) and bone disease (61%), and number of sites of metastases (greater than two: 50%), were also similar in the HALAVEN and control arms. Patients received a median of four prior chemotherapy regimens in both arms.

In Study 1, a statistically significant improvement in overall survival was observed in patients randomized to the HALAVEN arm compared to the control arm (see Table 3). An updated, unplanned survival analysis, conducted when 77% of events had been observed (see Figure 1), was consistent with the primary analysis. In patients randomized to HALAVEN, the objective response rate by the RECIST criteria was 11% (95% CI: 8.6%, 14.3%) and the median response duration was 4.2 months (95% CI: 3.8, 5.0 months).

Table 3 Comparison of Overall Survival in HALAVEN and Control Arm - Study 1

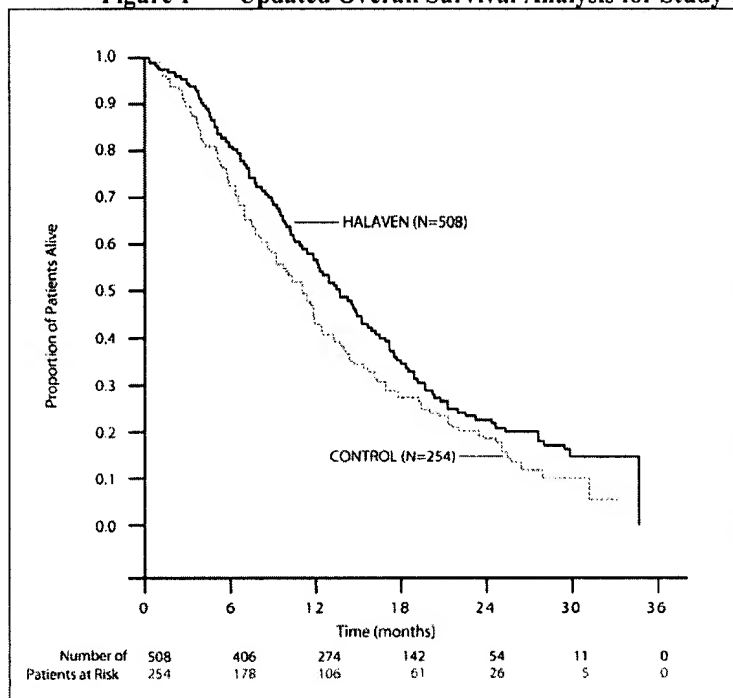
Overall Survival	HALAVEN (n=508)	Control Arm (n=254)
Primary survival analysis		
Number of deaths	274	148
Median, months (95% CI)	13.1 (11.8, 14.3)	10.6 (9.3, 12.5)
Hazard Ratio (95% CI) ^a	0.81 (0.66, 0.99)	
<i>P</i> value ^b	0.041	
Updated survival analysis		
Number of deaths	386	203
Median, months (95% CI)	13.2 (12.1, 14.4)	10.6 (9.2, 12.0)

CI = confidence interval

^a Based on Cox proportional hazards model stratified by geographic region, HER2 status, and prior capecitabine therapy.

^b Based on a log-rank test stratified by geographic region, HER2 status, and prior capecitabine therapy.

Figure 1 Updated Overall Survival Analysis for Study 1



16 HOW SUPPLIED/STORAGE AND HANDLING

NDC 62856-389-01 Eribulin mesylate injection, 1 mg/2 mL, in a single-use vial. One vial per carton.

Store at 25°C (77°F); excursions permitted to 15° – 30° C (59° -86° F). Do not freeze. Store the vials in their original cartons.

17 PATIENT COUNSELING INFORMATION

See FDA-Approved Patient Labeling

- Advise patients to contact their health care provider for a fever of 100.5°F or greater or other signs or symptoms of infection such as chills, cough, or burning or pain on urination. [see *Warnings and Precautions* (5.1)]
- Advise women of childbearing potential to avoid pregnancy and to use effective contraception during treatment with HALAVEN. [see *Warnings and Precautions* (5.3) and *Use in Specific Populations* (8.1)]

Manufactured by:
NerPharMa
Viale Pasteur, 10
20014, Nerviano
Italy

Distributed by:
Eisai Inc.
100 Tice Blvd. Woodcliff Lake, NJ 07677

PATIENT INFORMATION

HALAVEN™ (HAL-ih-ven) (eribulin mesylate) Injection

Read this leaflet before you start receiving HALAVEN and before each injection. There may be new information. This information does not take the place of talking with your healthcare provider about your medical condition or your treatment.

What is the most important information I should know about HALAVEN?

Your healthcare provider should do blood tests regularly to check your blood cell counts before you receive each dose of HALAVEN.

- HALAVEN can cause a decrease in white blood cell count (neutropenia). This can make you more likely to get serious infections that could lead to death. You may need treatment in the hospital with antibiotic medicines.
- Call your healthcare provider right away if you develop any of these symptoms of infection while you are receiving HALAVEN:
 - fever (temperature above 100.5°F)
 - chills
 - cough
 - burning or pain when you urinate.
- HALAVEN can cause numbness, tingling, or burning in your hands and feet (neuropathy). Tell your healthcare provider if you have any of these symptoms.

See **"What are possible side effects of HALAVEN?"** for more information about side effects.

What is HALAVEN?

HALAVEN is a prescription medicine used to treat people with breast cancer:

- that has spread to other parts of their body, and
- who have already received certain types of anticancer medicines after their breast cancer has spread.

What should I tell my healthcare provider before receiving HALAVEN?

Before you receive HALAVEN, tell your healthcare provider if you:

- have liver or kidney problems.
- have heart problems, including a problem called "congenital long QT syndrome."

- are pregnant or plan to become pregnant. **HALAVEN may harm your unborn baby.** Talk with your healthcare provider about birth control methods to prevent pregnancy while you receive HALAVEN. Tell your healthcare provider right away if you become pregnant or think you are pregnant while you are receiving HALAVEN
- are breastfeeding or planning to breastfeed. It is not known if HALAVEN passes into your breast milk. You and your healthcare provider should decide if you will take HALAVEN or breastfeed. You should not do both.

Tell your healthcare provider about all the medicines you take, including prescription and non-prescription medicines, vitamins and herbal supplements.

Know the medicines you take. Keep a list of your medicines to show to your healthcare provider and pharmacist when you get a new medicine.

How will I receive HALAVEN?

- HALAVEN is injected directly into your vein.
- HALAVEN is given in "cycles" of treatment, with each cycle lasting 21 days.
- You will receive an injection 1 time each week for two weeks in a row (day 1 and day 8 of a treatment cycle).
- Your healthcare provider may need to decrease your dose of HALAVEN or change how often you receive it, depending on your blood test results.

What are the possible side effects of HALAVEN?

HALAVEN may cause serious side effects, including:

- See "**What is the most important information I should know about HALAVEN?**"
- **HALAVEN can cause changes in your heartbeat (called QTc prolongation).** This can cause irregular heartbeats that may lead to death. Your healthcare provider will decide if you need heart monitoring (electrocardiogram or ECG), or blood tests during your treatment with HALAVEN to watch for this problem.

The most common side effects of HALAVEN include:

- weakness or tiredness
- hair loss
- nausea
- constipation

Tell your healthcare provider about any side effect that bothers you or that does not go away.

These are not all the possible side effects of HALAVEN. For more information, ask your healthcare provider or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

General information about HALAVEN

Medicines are sometimes prescribed for purposes other than those listed in a patient information leaflet. This leaflet summarizes the most important information about HALAVEN. If you would like more information, **talk with your healthcare provider**. You can ask your pharmacist or healthcare provider for information about HALAVEN that is written for health professionals.

For more information, go to www.HALAVEN.com or call Eisai Inc. at 1-877-873-4724.

What are the ingredients in HALAVEN?

Active Ingredients: eribulin mesylate

Inactive Ingredients: ethanol, water

Distributed by:

Eisai Inc.

100 Tice Blvd. Woodcliff Lake, NJ 07677

If you would like a leaflet with larger printing, please contact Eisai Inc. at 1-877-873-4724.

EXHIBIT 4



**DEPARTMENT OF HEALTH AND HUMAN
SERVICES**

**Food and Drug
Administration Silver
Spring MD 20993**

NDA 201532

NDA APPROVAL

Eisai, Incorporated
Attention: Annmarie Petraglia
Senior Director, Global Regulatory Affairs
300 Tice Boulevard
Woodcliff Lake, NJ 07677

Dear Ms. Petraglia:

Please refer to your New Drug Application (NDA) dated March 30, 2010, received March 30, 2010, submitted under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act (FDCA) for Halaven (eribulin mesylate) Injection, 0.5mg/mL (1.0 mg/2 mL solution).

Please also refer to our original action letter dated November 15, 2010 approving your NDA application for Halaven (eribulin mesylate) Injection and to your electronic mail communication dated November 16, 2010, notifying us that the dates specified in the timetable for postmarketing requirement (PMR) 1689-1 were incorrect. This replacement action letter incorporates the correct timetable dates for PMR 1689-1. The effective action date will remain November 15, 2010, the date of the original action letter.

We acknowledge receipt of your amendments dated March 30, April 2, May 11, May 17, May 21, June 7, June 17, June 22, June 28, June 29, July 23, July 28 (2), August 9 (2), August 12, September 10 (2), September 16, September 23, September 24, September 28, September 30 (2), October 6, October 13, October 14, October 22, October 27, October 29 and November 11, 2010.

This new drug application provides for the use of Halaven (eribulin mesylate) Injection for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting.

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the enclosed agreed-upon labeling text.

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, submit, via the FDA automated drug registration and listing system (eLIST), the content of labeling [21 CFR 314.50(l)] in structured product labeling (SPL) format, as described at <http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm>, that is identical to the enclosed labeling (package insert, patient information) Information on submitting SPL files using eLIST may be found in the guidance for industry titled "SPL Standard for Content of Labeling Technical Qs and As" at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf>.

The SPL will be accessible via publicly available labeling repositories.

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed carton and immediate container labels as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled "Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008)." Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Final Printed Carton and Container Labels for approved NDA 201532.**" Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

ADVISORY COMMITTEE

Your application for Halaven was not referred to an FDA advisory committee because there were no controversial issues that would have benefitted from advisory committee discussion.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are waiving the pediatric study requirement for this application because necessary studies are impossible or highly impracticable since breast cancer is rare in the 0-18 year old age group.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o)(3) of the FDCA authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to identify an unexpected, serious risk of increased toxicity in patients with impaired renal function.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA has not yet been established and is not sufficient to assess these serious risks.

Finally, we have determined that only a clinical trial (rather than a nonclinical or observational study) will be sufficient to identify an unexpected serious risk of increased toxicity in patients with impaired renal function.

Therefore, based on appropriate scientific data, FDA has determined that you are required, to conduct the following:

PMR 1689-1:

To conduct a dedicated clinical trial assessing the safety and pharmacokinetics of Halaven, in accordance with FDA Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function - Study Design, Data Analysis and Impact on Dosing and Labeling. The trial design should include subjects with normal renal function and subjects with severe renal impairment.

The study population may include patients with advanced or metastatic solid tumors that are no longer responding to available therapy, i.e., similar eligibility criteria with regard to cancer type as for Trial 108 conducted in cancer patients with hepatic impairment. The renal function subgroups should have similar demographic characteristics with respect to age, gender and weight. The number of patients enrolled in the trial should be sufficient to detect clinically important PK differences that would warrant dosage adjustment recommendation. The frequency and duration of plasma sampling should be sufficient to accurately estimate relevant PK parameters for the parent drug. A data analysis plan should be included in the final protocol submitted to FDA.

The timetable you submitted on September 30, 2010 states that you will conduct this trial according to the following schedule:

Final Protocol Submission:	February 28, 2011
Trial Completion Date:	September 30, 2012
Final Report Submission:	March 31, 2013

Submit the protocol to IND 67193, with a cross-reference letter to this NDA. Submit all final report(s) to your NDA. Prominently identify the submission with the following wording in bold

capital letters at the top of the first page of the submission, as appropriate: **“Required Postmarketing Protocol Under 505(o)”**, **“Required Postmarketing Final Report Under 505(o)”**, **“Required Postmarketing Correspondence Under 505(o)”**.

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 314.81(b)(2)(vii) requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 314.81(b)(2)(vii) to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 314.81(b)(2)(vii). We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitments in your submission dated September 30, 2010. These commitments are listed below.

PMC 1689-2:

To submit a final report that includes updated results for overall survival after 95% of patient deaths have occurred (724 deaths in 762 enrolled patients) for trial E7389-G000-305, “A Phase 3 Open Label, Randomized Parallel Two-Arm Multi-Center Study of E7389 versus ‘Treatment of Physician’s Choice’ in Patients with Locally Recurrent or Metastatic Breast Cancer, Previously Treated with At Least Two and a Maximum of Five Prior Chemotherapy Regimens, Including an Anthracycline and a Taxane”. The final report should also include the primary and derived datasets and analysis programs used to generate the overall survival results reported..

The original protocol for clinical trial E7389-G000-305 was submitted to FDA on April 26, 2006, and began patient accrual on November 16, 2006. We also acknowledge receipt of the protocol amendments received on August 8, 2006; January 4, 2008; June 5, 2008; and March 3, 2009.

The timetable you submitted on September 30, 2010 states that you will conduct the trial according to the following schedule:

Final Report Submission:

March 1, 2013

PMC 1689-3:

To submit a final report for the ongoing trial, E7389-G000-301, "A Phase III Open Label, Randomized Two-Parallel-Arm Multicenter Study of E7389 versus Capecitabine in Patients with Locally Advanced or Metastatic Breast Cancer Previously Treated with Anthracyclines and Taxanes." This report will include a subset analysis of overall survival in patients that progressed while on treatment with a taxane or other microtubule inhibiting agent, in addition to all protocol-specified analyses. The original protocol for clinical trial E7389-G000-301 was submitted to FDA on November 17, 2005, and began patient accrual on September 20, 2006. We also acknowledge receipt of the protocol amendments received on December 14, 2005; March 2, 2006; May 11, 2006; December 5, 2006; October 31, 2007; March 6, 2008; and March 3, 2009.

The timetable you submitted on September 30, 2010 states that you will conduct the trial according to the following schedule:

Trial Completion Date:

March 31, 2012

Final Report Submission:

February 28, 2013

POSTMARKETING COMMITMENTS NOT SUBJECT TO THE REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of your postmarketing commitment in your submission dated September 17, 2010. This commitment is listed below.

PMC1689-4:

To provide a single Prior Approval Chemistry, Manufacturing and Controls (CMC) supplement containing all of the following data and information:

- Synthesis of the enantiomers of starting materials (b)(4); and analytical methods and acceptance criteria, with appropriate justification, specific to each enantiomer.
- Analytical methods and acceptance criteria with appropriate justification for Other Specified, Unspecified and Total Impurities in starting material (b)(4), and revised intermediates (b)(4)
- An identification test for intermediate (b)(4)
- Results of the evaluation for specificity of the current identification method for (b)(4) and, if necessary, develop a more selective method.
- More selective methods for identification and purity for the diastereomers of starting material (b)(4)

The timetable you submitted in the amendment dated September 17, 2010 states that you will submit the supplement according to the following schedule:

Final Report Submission:

March 31, 2011

Submit clinical protocols to IND 67193 for this product. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to this NDA. In addition, under 21 CFR 314.81(b)(2)(vii) and 314.81(b)(2)(viii) you should include a status summary of each commitment in your annual report to this NDA. The status summary should include expected summary completion and final report submission dates, any changes in plans since the last annual report, and, for clinical studies/trials, number of patients entered into each study/trial. All submissions, including supplements, relating to these postmarketing commitments should be prominently labeled “**Postmarketing Commitment Protocol**,” “**Postmarketing Commitment Final Report**,” or “**Postmarketing Commitment Correspondence**.”

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications
5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see <http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm>. Please submit one market package of the drug product when it is available.

LETTERS TO HEALTH CARE PROFESSIONALS

If you decide to issue a letter communicating important safety-related information about this drug product (i.e., a “Dear Health Care Professional” letter), we request that you submit, at least 24 hours prior to issuing the letter, an electronic copy of the letter to this NDA to the following address:

MedWatch Program
Office of Special Health Issues
Food and Drug Administration
10903 New Hampshire Ave
Building 32, Mail Stop 5353
Silver Spring, MD 20993

REPORTING REQUIREMENTS

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

MEDWATCH-TO-MANUFACTURER PROGRAM

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at <http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm>.

POST-ACTION FEEDBACK MEETING

New molecular entities and new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during drug development and marketing application review. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, call the Regulatory Project Manager for this application.

If you have any questions, call Vaishali Jarral, Regulatory Project Manager, at (301) 796-4248.

Sincerely,

{See appended electronic signature page}

Richard Pazdur, M.D.
Director
Office of Oncology Drug Products
Center for Drug Evaluation and Research

ENCLOSURES:

Content of Labeling [including Patient Labeling (PPI)]
Carton and Container Labeling

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD PAZDUR
11/15/2010

EXHIBIT 5



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 78859

ISTMT

DATE PRINTED
12/22/2010

Clark & Elbing LLP / Eisai
101 Federal Street
Suite 1500
Boston MA 02110

MAINTENANCE FEE STATEMENT

According to the records of the U.S. Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O. Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,214,865	\$940.00	\$0.00	10/07/04	09/334,488	04/10/01	06/16/99	04	NO	04520/016002



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The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O. Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,214,865	\$2,360.00	\$0.00	04/16/08	09/334,488	04/10/01	06/16/99	08	NO	04520/016002

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Shopping
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**United States
Patent and
Trademark Office**

Patent Bibliographic Data				01/04/2011 07:36 PM	
Patent Number:	6214865		Application Number:	09334488	
Issue Date:	04/10/2001		Filing Date:	06/16/1999	
Title:	MACROCYCLIC ANALOGS AND METHODS OF THEIR USE AND PREPARATION				
Status:	12th year fee window opens: 04/10/2012			Entity:	Large
Window Opens:	04/10/2012	Surcharge Date:	10/11/2012	Expiration:	N/A
Fee Amt Due:	Window not open	Surchg Amt Due:	Window not open	Total Amt Due:	Window not open
Fee Code:	1553	MAINTENANCE FEE DUE AT 11.5 YEARS			
Surcharge Fee Code:					
Most recent events (up to 7):	04/16/2008 Payment of Maintenance Fee, 8th Year, Large Entity. 10/07/2004 Payment of Maintenance Fee, 4th Year, Large Entity. --- End of Maintenance History ---				
Address for fee purposes:	Clark & Elbing LLP / Eisai 101 Federal Street Suite 1500 Boston, MA 02110				
<input type="button" value="Run Another Query"/>					

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EXHIBIT 6



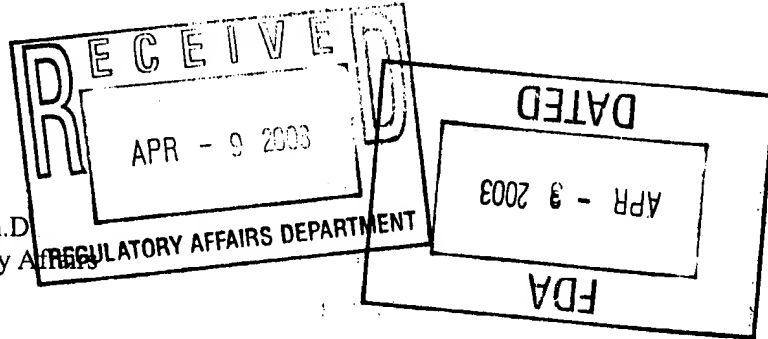
DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 67,193

Eisai Medical Research Inc.
Attention: Kathryn Bishburg, Pharm.D.
VP, Quality Assurance & Regulatory Affairs
Glenpointe Centre West
500 Frank W. Burr Blvd.
Teaneck, NJ 07666



Dear Dr. Bishburg:

We acknowledge receipt of your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned:	67,193
Sponsor:	Eisai Medical Research Inc.
Name of Drug:	E7389 (Halichondrin B analog) Injection
Date of Submission:	March 31, 2003
Date of Receipt:	March 31, 2003

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before April 30, 2003, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports [21 CFR 312.33].

Please forward all future communications concerning this IND in triplicate, identified by the above IND number, to either of the following addresses:

U.S. Postal Service:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products, HFD-150
Attention: Division Document Room, 3067
5600 Fishers Lane
Rockville, Maryland 20857

Courier/Overnight Mail:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products, HFD-150
Attention: Division Document Room, 3067
1451 Rockville Pike
Rockville, Maryland 20852

If you have any questions, call Patty Garvey, Regulatory Project Manager, at 301-594-5766.

Sincerely,

{See appended electronic signature page}

Dotti Pease
Chief, Project Management Staff
Division of Oncology Drug Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Patricia Garvey
4/3/03 02:25:25 PM
Signed for Dotti Pease

EXHIBIT 7



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 201532

PRIORITY REVIEW DESIGNATION

Eisai, Incorporated
Attention: Annmarie Petraglia
Senior Director, Global Regulatory Affairs
300 Tice Boulevard
Woodcliff Lake, NJ 07677

Dear Ms. Petraglia:

Please refer to your new drug application (NDA) dated March 30, 2010, received March 30, 2010, submitted under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act, for "Eribulin Mesylate, Injection, 0.5mg/mL (1.0 mg/2mL solution)."

We have completed our filing review and have determined that your application is sufficiently complete to permit a substantive review. Therefore, this application is considered filed 60 days after the date we received your application in accordance with 21 CFR 314.101(a). The review classification for this application is **Priority**. Therefore, the user fee goal date is September 30, 2010.

We are reviewing your application according to the processes described in the Guidance for Review Staff and Industry: Good Review Management Principles and Practices for PDUFA Products. Therefore, we have established internal review timelines as described in the guidance, which includes the timeframes for FDA internal milestone meetings (e.g., filing, planning, mid-cycle, team and wrap-up meetings). Please be aware that the timelines described in the guidance are flexible and subject to change based on workload and other potential review issues (e.g., submission of amendments). We will inform you of any necessary information requests or status updates following the milestone meetings or at other times, as needed, during the process. If major deficiencies are not identified during the review, we plan to communicate proposed labeling and, if necessary, any postmarketing commitment requests or postmarketing requirements by September 2, 2010.

While conducting our filing review, we identified potential review issues and will communicate them to you on or before June 11, 2010.

If you have any questions, call Vaishali Jarra, Regulatory Project Manager, at (301) 796-4248

Sincerely,

{See appended electronic signature page}

Patricia Keegan, M.D.
Director
Office of Oncology Drug Products
Division of Oncology Biologic Products
Center for Drug Evaluation and Research

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-201532	ORIG-1	EISAI INC	eribulin mesylate

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

VAISHALI JARRAL
05/26/2010

PATRICIA KEEGAN
05/27/2010

EXHIBIT 8

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
03/31/03	Original Submission	Original IND
04/09/03	Incoming FDA Correspondence	FDA Correspondence
04/23/03	TCR	FDA Correspondence
04/25/03	Incoming FDA Correspondence	FDA Correspondence
04/28/03	Incoming FDA Correspondence	FDA Correspondence
04/29/03	TCR	FDA Correspondence
04/29/03	Information Amendment	Information Amendment: Response to FDA Request for Information
04/30/03	Incoming FDA Correspondence	FDA Correspondence
06/03/03	Safety Report	IND Safety Report
06/10/03	Protocol Amendment	Protocol Amendment: Change In Protocol
06/20/03	Incoming FDA Correspondence	FDA Correspondence
06/25/03	Protocol Amendment	Protocol Amendment: New Protocol/New Investigator
07/10/03	Incoming FDA Correspondence	FDA Correspondence
07/14/03	Incoming FDA Correspondence	FDA Correspondence
07/22/03	Protocol Amendment	Protocol Amendment: New Investigator
07/31/03	Information Amendment	Information Amendment: Pharmacology / Toxicology
09/11/03	Incoming FDA Correspondence	FDA Correspondence
09/22/03	Protocol Amendment	Protocol Amendment: Change In Protocol
09/22/03	Protocol Amendment	Protocol Amendment: Change In Protocol
12/12/03	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
12/18/03	Safety Report	IND Safety Report
01/12/04	Safety Report	IND Safety Report
02/04/04	Safety Report	IND Safety Report
02/23/04	General Correspondence	General Correspondence: Change of Sponsor Address
02/26/04	Safety Report	IND Safety Report
03/11/04	Safety Report	IND Safety Report
03/12/04	Protocol Amendment	Protocol Amendment: Change in Protocol
04/07/04	Information Amendment	Information Amendment: CMC
04/19/04	Safety Report	IND Safety Report
04/21/04	Safety Report	IND Safety Report
04/28/04	Safety Report	IND Safety Report
05/03/04	Safety Report	IND Safety Report
05/07/04	Information Amendment	Information Amendment: Pharmacology/Toxicology
05/17/04	Safety Report	IND Safety Report
05/26/04	Annual Report	Annual Report: 3/31/03 - 3/30/04
05/28/04	Safety Report	IND Safety Report
06/08/04	Safety Report	IND Safety Report
06/19/04	Safety Report	IND Safety Report
07/28/04		Protocol Amendment: Updated Investigator Information
08/27/04	Safety Report	IND Safety Report
09/03/04	Safety Report	IND Safety Report
09/16/04	Safety Report	IND Safety Report
09/17/04	Protocol Amendment	Protocol Amendment: New Protocol/New Investigator
09/22/04	Safety Report	IND Safety Report
09/27/04	Safety Report	IND Safety Report
10/19/04	Safety Report	IND Safety Report
11/02/04	Safety Report	IND Safety Report
11/08/04	Safety Report	IND Safety Report
11/12/04	Safety Report	IND Safety Report
11/18/04	Protocol Amendment	Protocol Amendment: New Investigators
11/24/04	Safety Report	IND Safety Report
12/02/04	Safety Report	IND Safety Report

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
12/14/04	Protocol Amendment	Protocol Amendment: New Protocol/New Investigator
12/14/04	Safety Report	IND Safety Report
12/15/04	Safety Report	IND Safety Report
12/16/04	Safety Report	IND Safety Report
12/22/04	Protocol Amendment	Protocol Amendment; New Investigators
01/11/05	Safety Report	IND Safety Report
01/19/05	Safety Report	IND Safety Report
01/27/05	Protocol Amendment	Protocol Amendment: New Investigator
01/27/05	Safety Report	IND Safety Report
02/01/05	Safety Report	IND Safety Report
02/03/05	Safety Report	IND Safety Report
02/10/05	Safety Report	IND Safety Report
02/15/05	Safety Report	IND Safety Report
02/17/05	Safety Report	IND Safety Report
02/22/05	Safety Report	IND Safety Report
03/02/05	Safety Report	IND Safety Report
03/09/05	Safety Report	IND Safety Report
03/15/05	Safety Report	IND Safety Report
03/18/05	Safety Report	IND Safety Report
03/24/05	Safety Report	IND Safety Report
04/01/05	Safety Report	IND Safety Report
04/04/05	Safety Report	IND Safety Report
04/04/05	Safety Report	IND Safety Report
04/05/05	Safety Report	IND Safety Report
04/07/05	Safety Report	IND Safety Report
04/07/05	Safety Report	IND Safety Report
04/08/05	Safety Report	IND Safety Report
04/08/05	Safety Report	IND Safety Report
04/14/05	Safety Report	IND Safety Report
04/15/05	Safety Report	IND Safety Report
04/20/05	Safety Report	IND Safety Report
04/21/05	Protocol Amendment	Protocol Amendment: New Investigator
04/22/05	Safety Report	IND Safety Report
04/28/05	Safety Report	IND Safety Report
05/03/05	Safety Report	IND Safety Report
05/04/05	Safety Report	IND Safety Report
05/12/05	Protocol Amendment	Protocol Amendment: New Investigator
05/20/05	Safety Report	IND Safety Report
05/26/05	Safety Report	IND Safety Report
05/31/05	Annual Report	Annual Report: 3/31/04 - 3/30/05
06/02/05	Safety Report	IND Safety Report
06/03/05	Safety Report	IND Safety Report
06/06/05	Safety Report	IND Safety Report
06/16/05	Safety Report	IND Safety Report
06/20/05	Request for Meeting	Request for Meeting: Type B (End of Phase 2)
06/22/05	Safety Report	IND Safety Report
06/24/05	Information Amendment	Information Amendment: Pharmacology/Toxicology
06/29/05	Safety Report	IND Safety Report
06/30/05	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
06/30/05	Safety Report	IND Safety Report
07/06/05	Safety Report	IND Safety Report
07/12/05	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
07/13/05	Safety Report	IND Safety Report
07/14/05	Safety Report	IND Safety Report
07/15/05	Safety Report	IND Safety Report
07/18/05	Safety Report	IND Safety Report
07/19/05		IND Safety Report
07/20/05	Safety Report	IND Safety Report
07/21/05	Safety Report	IND Safety Report
07/25/05	Safety Report	IND Safety Report
07/28/05	Safety Report	IND Safety Report
08/01/05	Safety Report	IND Safety Report
08/03/05	Safety Report	IND Safety Report
08/05/05	Briefing Package	Briefing Package for End-of-Phase 2 Meeting
08/08/05	Safety Report	IND Safety Report
08/12/05	Safety Report	IND Safety Report
08/16/05	Safety Report	IND Safety Report
08/23/05	Safety Report	IND Safety Report
08/26/05	Safety Report	IND Safety Report
08/30/05	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
09/02/05	Incoming FDA Correspondence	FDA Correspondence
09/06/05	TCR	FDA Correspondence
09/09/05	Safety Report	IND Safety Report
09/15/05	Incoming FDA Correspondence	FDA Correspondence
09/15/05	Safety Report	IND Safety Report
09/16/05	Protocol Amendment	Protocol Amendment: Updated Investigator
09/20/05	Safety Report	IND Safety Report
10/05/05	Safety Report	IND Safety Report
10/13/05	Safety Report	IND Safety Report
10/19/05	Information Amendment	Information Amendment: CMC
10/20/05	Protocol Amendment	Protocol Amendment: New Protocol/New Investigator
10/20/05	Safety Report	IND Safety Report
10/27/05	Safety Report	IND Safety Report
10/28/05	Safety Report	IND Safety Report
11/01/05	Safety Report	IND Safety Report
11/03/05	Safety Report	IND Safety Report
11/09/05	Safety Report	IND Safety Report
11/15/05	Safety Report	IND Safety Report
11/22/05	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
11/22/05	Safety Report	IND Safety Report
12/16/05	Request for Special Assessment	Request for Special Protocol Assessment: Clinical Protocol
12/16/05	Safety Report	IND Safety Report
12/20/05	Safety Report	IND Safety Report
12/21/05	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
01/17/06	Protocol Amendment	Protocol Amendment: New Protocol
01/18/06	Safety Report	IND Safety Report
01/20/06	Safety Report	IND Safety Report
01/26/06	Response to FDA Request	Response to FDA 9/2/05 Request for Information
01/30/06	Safety Report	IND Safety Report
02/02/06	Request for Meeting	Meeting Request: End-of-Phase 2 CMC (Type B)
02/08/06	Safety Report	IND Safety Report
02/14/06	Safety Report	IND Safety Report
02/23/06	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
02/24/06	Safety Report	IND Safety Report

HALAVEN™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
03/01/06	Safety Report	IND Safety Report
03/10/06	Briefing Package	Briefing Package for End-of-Phase 2 CMC Meeting
03/16/06	Protocol Amendment	New Investigator/Updated Investigator
03/16/06	Response to FDA Request	Sample Case Report Forms
03/30/06	Safety Report	IND Safety Report
03/30/06	Safety Report	IND Safety Report
03/31/06	Safety Report	IND Safety Report
04/06/06	Response to FDA Request	Response to FDA Fax of 3/7/06
04/07/06	Safety Report	IND Safety Report
04/13/06	Safety Report	IND Safety Report
04/14/06	Safety Report	IND Safety Report
04/19/06	Protocol Amendment	Protocol Amendment: New Investigators/Updated Investigators
04/21/06	Safety Report	IND Safety Report
04/27/06	Safety Report	IND Safety Report
05/01/06	Safety Report	IND Safety Report
05/11/06	Safety Report	IND Safety Report
05/15/06	Safety Report	IND Safety Report
05/17/06	Safety Report	IND Safety Report
05/17/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
05/18/06	Safety Report	IND Safety Report
05/23/06	Safety Report	IND Safety Report
05/25/06	Information Amendment	Pharm/Tox
05/26/06	Safety Report	IND Safety Report
05/30/06	Protocol Amendment	Protocol Amendment
06/01/06	Safety Report	IND Safety Report
06/01/06	Annual Report	Annual Report: 3/31/05 - 3/30/06
06/05/06	Safety Report	IND Safety Report
06/06/06	Safety Report	IND Safety Report
06/07/06	Safety Report	IND Safety Report
06/12/06	Safety Report	IND Safety Report
06/13/06	Safety Report	IND Safety Report
06/15/06	Safety Report	IND Safety Report
06/20/06	Safety Report	IND Safety Report
06/21/06	Safety Report	IND Safety Report
06/21/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
06/23/06	Safety Report	IND Safety Report
06/27/06	Safety Report	IND Safety Report
06/29/06	Request for Meeting	Meeting request and briefing book
07/05/06	Safety Report	IND Safety Report
07/06/06	Safety Report	IND Safety Report
07/07/06	Information Amendment	Information Amendment: Investigator's Brochure
07/10/06	Safety Report	IND Safety Report
07/13/06	Safety Report	IND Safety Report
07/18/06	Safety Report	IND Safety Report
07/24/06	Safety Report	IND Safety Report
07/25/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
07/27/06	Information Amendment	Information Amendment: CMC
07/28/06	Safety Report	IND Safety Report
07/31/06	Safety Report	IND Safety Report
08/02/06	Safety Report	IND Safety Report
08/08/06	Safety Report	IND Safety Report
08/14/06	Safety Report	IND Safety Report

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
08/17/06	Safety Report	IND Safety Report
08/17/06	Safety Report	IND Safety Report
08/21/06	Safety Report	IND Safety Report
08/22/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
08/24/06	Safety Report	IND Safety Report
08/28/06	Protocol Amendment	BioImaging Charter
08/29/06	Safety Report	IND Safety Report
09/05/06	Safety Report	IND Safety Report
09/12/06	Safety Report	IND Safety Report
09/14/06	Safety Report	IND Safety Report
09/18/06	Safety Report	IND Safety Report
09/21/06	Protocol Amendment	Protocol Amendment: New Protocol
09/27/06	Safety Report	IND Safety Report
09/28/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
10/04/06	Safety Report	IND Safety Report
10/04/06	Safety Report	IND Safety Report
10/05/06	Safety Report	IND Safety Report
10/11/06	Safety Report	IND Safety Report
10/11/06	Protocol Amendment	Protocol Amendment: New Protocol/New Investigator
10/17/06	Safety Report	IND Safety Report
10/18/06	Safety Report	IND Safety Report
10/20/06	Safety Report	IND Safety Report
10/20/06	Safety Report	IND Safety Report
10/23/06	Safety Report	IND Safety Report
10/24/06	Safety Report	IND Safety Report
10/26/06	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
10/30/06	Safety Report	IND Safety Report
11/01/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
11/02/06	Safety Report	IND Safety Report
11/06/06	Safety Report	IND Safety Report
11/08/06	Safety Report	IND Safety Report
11/09/06	Safety Report	IND Safety Report
11/13/06	Safety Report	IND Safety Report
11/15/06	Safety Report	IND Safety Report
11/21/06	Safety Report	IND Safety Report
11/21/06	Request for Meeting	Request for second End-of-Phase 2 Meeting
11/27/06	Safety Report	IND Safety Report
11/28/06	Safety Report	IND Safety Report
12/05/06	E-Mail	FDA correspondence dated 12/4/06
12/05/06	Safety Report	IND Safety Report
12/06/06	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
12/07/06	Fax	1/30/07 End-of-Phase 2 Meeting
12/08/06	Safety Report	IND Safety Report
12/12/06	Safety Report	IND Safety Report
12/14/06	Safety Report	IND Safety Report
12/14/06	Protocol Amendment	Protocol Amendment: Change in Protocol
12/18/06	Safety Report	IND Safety Report
12/18/06	Protocol Amendment	Protocol Amendment: Change in Protocol
12/19/06	Fax	Comments on IRC Charter
12/21/06	Safety Report	IND Safety Report
12/21/06	Briefing Package	End-of-Phase 2 Meeting
12/26/06	Safety Report	IND Safety Report

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
12/28/06	Safety Report	IND Safety Report
01/02/07	Safety Report	IND Safety Report
01/04/07	E-Mail	Amendment 04—SPA
01/04/07	Safety Report	IND Safety Report
01/04/07	Safety Report	IND Safety Report
01/09/07	E-Mail	FDA email correspondence
01/09/07	Safety Report	IND Safety Report
01/11/07	Safety Report	IND Safety Report
01/12/07	E-Mail	Imaging Charter
01/17/07	Safety Report	IND Safety Report
01/22/07	E-Mail	FDA Email
01/22/07	Fax	FDA Response to End-of-Phase2 Meeting Briefing Package Questions
01/22/07	E-Mail	FDA email for questions in electronic format
01/22/07	E-Mail	Response to 1/22/07 FDA email
01/22/07	Safety Report	IND Safety Report
01/23/07	TCR	FDA Telephone Contact
01/29/07	Incoming FDA Correspondence	Acknowledgement Receipt of SPA
01/29/07	Fax	FDA Fax Dated 1/29/07
01/29/07	TCR	Imaging Charter
01/29/07	Safety Report	IND Safety Report
02/01/07	Safety Report	IND Safety Report
02/07/07	Incoming FDA Correspondence	FDA Response to Amendment—SPA
02/08/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
02/12/07	Safety Report	IND Safety Report
02/13/07	Safety Report	IND Safety Report
02/13/07	Safety Report	IND Safety Report
02/15/07	TCR	FDA Response to Revision of Amendment—SPA
02/16/07	Information Amendment	Information Amendment: Clinical
02/23/07	Protocol Amendment	Response to FDA Comments on Imaging Review Charter
02/26/07	Safety Report	IND Safety Report
02/27/07	Protocol Amendment	BioImaging Charter and Censoring Rules
03/01/07	Safety Report	IND Safety Report
03/05/07	E-Mail	MedDRA version
03/08/07	Protocol Amendment	SPA
03/12/07	Safety Report	IND Safety Report
03/14/07	Safety Report	IND Safety Report
03/15/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
03/21/07	Safety Report	IND Safety Report
03/23/07	TCR	FDA Correspondence
03/26/07	Safety Report	IND Safety Report
03/27/07	E-Mail	FDA Correspondence on Ongoing Study Data Requirements for NDA Filing
03/29/07	Protocol Amendment	Protocol Amendment: Change in Protocol
04/02/07	Safety Report	IND Safety Report
04/06/07	E-Mail	FDA Comments on Imaging Charter
04/06/07	Safety Report	IND Safety Report
04/09/07	E-Mail	FDA Correspondence: Comments on Imaging Charter
04/12/07	Safety Report	IND Safety Report
04/18/07	Safety Report	IND Safety Report
04/19/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
04/25/07	General Correspondence	Request for trade name
04/26/07	Safety Report	IND Safety Report

HALAVEN™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
05/01/07	Safety Report	IND Safety Report
05/07/07	Incoming FDA Correspondence	Comments on Submission of SPA
05/07/07	Safety Report	IND Safety Report
05/10/07	Safety Report	IND Safety Report
05/18/07	Safety Report	IND Safety Report
05/21/07	Fax	FDA correspondence
05/22/07	Fax	FDA Correspondence
05/22/07	Protocol Amendment	Protocol Amendment: New Investigator
05/25/07	Safety Report	IND Safety Report
05/30/07	Annual Report	Annual Report
06/04/07	Safety Report	IND Safety Report
06/08/07	Request for Meeting	Request for Pre-NDA Meeting
06/11/07	E-Mail	FDA Correspondence: Global Safety Reporting
06/12/07	E-Mail	FDA Correspondence: Pre-NDA Meeting date
06/14/07	Incoming FDA Correspondence	FDA Correspondence: Confirmation of Pre-NDA Meeting on 8/23/07
06/18/07	Safety Report	IND Safety Report
06/19/07	Safety Report	IND Safety Report
06/20/07	Safety Report	IND Safety Report
06/21/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
06/22/07	General Correspondence	Request for Fast Track Designation
06/26/07	Safety Report	IND Safety Report
06/27/07	Safety Report	IND Safety Report
07/03/07	Safety Report	IND Safety Report
07/05/07	E-Mail	Request For Teleconference To Discuss Imaging Charter and SAP
07/06/07	Response to FDA Request	Response to FDA Request for Information
07/09/07	Safety Report	IND Safety Report
07/10/07	Incoming FDA Correspondence	FDA Receipt of Fast Track Designation Request
07/12/07	TCR	Request For Teleconference To Discuss Imaging Charter, SAP, and Pre-NDA Meeting Briefing Documents
07/13/07	E-Mail	Response to 7/12/07 FDA Correspondence
07/13/07	E-Mail	FDA Responses to Charter
07/13/07	E-Mail	FDA Response to Request for Teleconference to discuss Imaging Charter
07/13/07	E-Mail	FDA Response Regarding Status of SAP and Charter
07/16/07	Safety Report	IND Safety Report
07/19/07	Safety Report	IND Safety Report
07/24/07	Briefing Package	Pre-NDA Meeting
07/25/07	Safety Report	IND Safety Report
07/26/07	Safety Report	IND Safety Report
07/30/07	E-Mail	Pre-NDA Meeting Briefing Package (document plus appendices)
07/30/07	E-Mail	7/31/07 Teleconference To Discuss Imaging Charter
07/30/07	Safety Report	IND Safety Report
07/31/07	Safety Report	IND Safety Report
07/31/07	Safety Report	IND Safety Report
08/01/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
08/06/07	Safety Report	IND Safety Report
08/07/07	Safety Report	IND Safety Report
08/08/07	Incoming FDA Correspondence	Preliminary FDA responses to questions
08/13/07	Safety Report	IND Safety Report
08/14/07	TCR	FDA Correspondence: Follow-up items

HALAVEN™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
08/14/07	Safety Report	IND Safety Report
08/15/07	E-Mail	FDA Correspondence
08/15/07	Protocol Amendment	New Protocol
08/20/07	E-Mail	FDA correspondence: Confirmation of Pre-NDA meeting
08/20/07	E-Mail	FDA correspondence: Pre-NDA Meeting Discussion Points
08/21/07	E-Mail	FDA Correspondence: Request for Slides
08/21/07	Safety Report	IND Safety Report
08/24/07	E-Mail	FDA Correspondence: Imaging Charter
08/27/07	Incoming FDA Correspondence	FDA Correspondence: Fast Track Designation
08/27/07	E-Mail	FDA Correspondence: Pre-NDA meeting minutes
08/28/07	Safety Report	IND Safety Report
08/30/07	Safety Report	IND Safety Report
08/30/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
09/04/07	Incoming FDA Correspondence	FDA Correspondence: Official Minutes of 7/31/07 Telecon
09/05/07	Safety Report	IND Safety Report
09/07/07	General Correspondence	Eisai's 8/23/07 Pre-NDA Meeting Minutes
09/11/07	Safety Report	IND Safety Report
09/14/07	Safety Report	IND Safety Report
09/17/07	Safety Report	IND Safety Report
09/20/07	Safety Report	IND Safety Report
09/21/07	Safety Report	IND Safety Report
09/21/07	Safety Report	IND Safety Report
09/26/07	E-Mail	FDA/CDER-edata group correspondence
09/27/07	Safety Report	IND Safety Report
10/02/07	Safety Report	IND Safety Report
10/04/07	Safety Report	IND Safety Report
10/04/07	Safety Report	IND Safety Report
10/08/07	Safety Report	IND Safety Report
10/10/07	Safety Report	IND Safety Report
10/11/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
10/12/07	Safety Report	IND Safety Report
10/17/07	TCR	FDA Correspondence
10/23/07	Safety Report	IND Safety Report
10/26/07	TCR	FDA correspondence
10/29/07	Safety Report	IND Safety Report
10/30/07	Information Amendment	Information Amendment: Pharm/Tox
10/31/07	TCR	Proposed changes to Special Protocol Assessment
10/31/07	Protocol Amendment	Proposed changes to Special Protocol Assessment
10/31/07	Information Amendment	Information Amendment: Investigator's Brochure
11/06/07	Safety Report	IND Safety Report
11/13/07	Safety Report	IND Safety Report
11/14/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
11/19/07	Safety Report	IND Safety Report
11/19/07	Safety Report	IND Safety Report
11/26/07	Safety Report	IND Safety Report
11/26/07	Safety Report	IND Safety Report
11/27/07	Protocol Amendment	Protocol Amendment: Change in Protocol
11/30/07	Safety Report	IND Safety Report
12/04/07	E-Mail	Email to FDA re CSR data listings
12/04/07	E-Mail	Acknowledgement of FDA comments
12/04/07	E-Mail	Comments on Proposed Study Changes
12/05/07	E-Mail	FDA Correspondence

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
12/06/07	Safety Report	IND Safety Report
12/07/07	E-Mail	FDA Correspondence
12/12/07	Safety Report	IND Safety Report
12/17/07	E-Mail	FDA Confirmation of 12/20/07 Telecon on Amendment 6 statistics
12/17/07	E-Mail	Confirmation of 12/14/07 Telecon Attendees
12/19/07	Safety Report	IND Safety Report
12/20/07	Safety Report	IND Safety Report
12/20/07	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
12/21/07	E-Mail	Special Protocol Assessment
12/21/07	E-Mail	FDA Confirmation of Study Stratification Factors
12/27/07	E-Mail	FDA Correspondence
12/27/07	Safety Report	IND Safety Report
01/02/08	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
01/03/08	Safety Report	IND Safety Report
01/07/08	Information Amendment	Information Amendment: Chemistry/Manufacturing/Controls
01/08/08	TCR	FDA Correspondence
01/08/08	Safety Report	IND Safety Report
01/14/08	E-Mail	Official Minutes from 12/14/07 Teleconference
01/14/08	E-Mail	Official Minutes from 12/20/07 Teleconference
01/15/08	Safety Report	IND Safety Report
01/23/08	Safety Report	IND Safety Report
01/30/08	Safety Report	IND Safety Report
01/31/08	TCR	FDA Correspondence
02/04/08	TCR	Meeting to discuss Study for breast cancer registration
02/05/08	E-Mail	Tentative FDA clearance of proposed trade names
02/06/08	Safety Report	IND Safety Report
02/06/08	Request for Meeting	Type C Meeting Request
02/13/08	E-Mail	Acceptance of 3/27/08 Meeting Date
02/13/08	E-Mail	3/27/08 End-of-Phase 2 Follow-up Meeting
02/14/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
02/14/08	Safety Report	IND Safety Report
02/15/08	E-Mail	New FDA RA Project Manager
02/15/08	Safety Report	IND Safety Report
02/20/08	E-Mail	Confirmation that meeting request is being processed
02/28/08	E-Mail	FDA Confirmation of Receipt of Questions (electronic version)
02/28/08	Briefing Package	3/27/08 End-of-Phase 2 Follow-up Meeting
03/03/08	E-Mail	3/27/08 End-of-Phase 2 Follow-up Meeting
03/05/08	Safety Report	IND Safety Report
03/18/08	Safety Report	IND Safety Report
03/19/08	TCR	Status of FDA response to questions for 3/27/08 End-of-Phase-2 Follow-up Meeting
03/19/08	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
03/21/08	E-Mail	FDA Responses for 3/27/08 End-of-Phase-2 Follow-up Meeting
03/25/08	Safety Report	IND Safety Report
03/27/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
04/02/08	Protocol Amendment	Amendment 6 (confirmation of SPA)
04/08/08	Safety Report	IND Safety Report
04/08/08	Response to FDA Request	Eisai Response to FDA Comments on 3/27/08 End-of-Phase-2 Follow-up Meeting
04/10/08	E-Mail	Notification of submission (SPA Amendment 6) and Response to FDA Comments on 3/27/08 End-of-Phase 2 Follow-up Meeting

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
04/15/08	TCR	FDA Correspondence
04/15/08	Safety Report	IND Safety Report
04/16/08	E-Mail	Confirmation of receipt of Protocol Amendment 6 SPA confirmation
04/17/08	TCR	Discussion of SPA Imaging Charter
04/21/08	Safety Report	IND Safety Report
04/23/08	Safety Report	IND Safety Report
04/28/08	General Correspondence	ICON Clinical Research
04/29/08	Safety Report	IND Safety Report
04/30/08	Safety Report	IND Safety Report
04/30/08	Safety Report	IND Safety Report
05/01/08	E-Mail	FDA Correspondence
05/01/08	General Correspondence	Response to FDA Request: SPA Summary of Independent Imaging Review Charter Changes
05/01/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
05/02/08	E-Mail	Confirmation of receipt of SPA Summary of Independent Imaging Review Charter Changes
05/07/08	Safety Report	SPA Summary of Independent Imaging Review Charter Changes
05/12/08	Safety Report	IND Safety Report
05/21/08	Incoming FDA Correspondence	FDA Correspondence: SPA Confirmation
05/29/08	Annual Report	Annual Report
05/29/08	Safety Report	IND Safety Report
06/09/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
06/10/08	Safety Report	IND Safety Report
06/13/08	Protocol Amendment	New Protocol
06/16/08	E-Mail	FDA Correspondence: List of Eisai Attendees on 6/11/08 Teleconference
06/16/08	E-Mail	FDA Correspondence: FDA Confirmation of Receipt of Eisai Attendee List
06/18/08	Safety Report	IND Safety Report
06/20/08	Protocol Amendment	Protocol Amendment
06/26/08	Safety Report	IND Safety Report
07/02/08	Protocol Amendment	Protocol Amendment
07/07/08	Safety Report	IND Safety Report
07/10/08	Safety Report	IND Safety Report
07/16/08	Safety Report	IND Safety Report
07/21/08	Safety Report	IND Safety Report
07/23/08	Protocol Amendment	Protocol Amendment: New Protocol
07/24/08	E-Mail	FDA Correspondence: Eisai request for official FDA minutes from 3/27/08 End-of-Phase 2 Follow-up Meeting
07/24/08	E-Mail	FDA Correspondence
07/24/08	Safety Report	IND Safety Report
07/25/08	E-Mail	FDA Correspondence: Provision of Investigator's Brochure
07/25/08	E-Mail	FDA Correspondence
07/28/08	E-Mail	FDA Correspondence
07/28/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
07/29/08	Safety Report	IND Safety Report
07/31/08	Safety Report	IND Safety Report
08/05/08	E-Mail	FDA Correspondence
08/05/08	FDA Correspondence	FDA Correspondence
08/05/08	E-Mail	New FDA Regulatory Project Manager assigned to E7389

HALAVEN™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
08/05/08	E-Mail	Request for FDA Minutes from 3/27/08 End-of-Phase 2 Follow-up Meeting
08/06/08	Safety Report	IND Safety Report
08/11/08	Safety Report	IND Safety Report
08/13/08	Safety Report	IND Safety Report
08/20/08	E-Mail	Request for Official Minutes from 3/27/08 End-of-Phase 2 Follow-up Meeting
08/27/08	IND Safety Report	IND Safety Report
09/03/08	E-Mail	FDA Correspondence
09/09/08	Safety Report	IND Safety Report
09/10/08	Safety Report	IND Safety Report
09/11/08	TCR	FDA Correspondence
09/12/08	Safety Report	IND Safety Report
09/16/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
09/16/08	Maintenance	Protocol Amendment: New Investigator/Updated Investigator
09/22/08	Safety Report	IND Safety Report
09/24/08	E-Mail	FDA Correspondence
00/25/08	Safety Report	IND Safety Report
09/30/08	Safety Report	IND Safety Report
10/06/08	E-Mail	FDA Correspondence: Request for 3/27/08 End-of-Phase 2 Follow-up Meeting Minutes
10/07/08	TCR	API Process Validation
10/07/08	E-Mail	IND 67,193
10/07/08	Safety Report	IND Safety Report
10/08/08	Safety Report	IND Safety Report
10/09/08	General Correspondence	Trade Name Request
10/13/08	Safety Report	IND Safety Report
10/21/08	Safety Report	IND Safety Report
10/24/08		FDA Correspondence
10/24/08	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
10/24/08	Maintenance	Protocol Amendment: New Investigator/Updated Investigator
10/27/08	Safety Report	IND Safety Report
10/28/08	Safety Report	IND Safety Report
10/29/08	Protocol Amendment	Protocol Amendment: Change in Protocol
10/29/08	Maintenance	Protocol Amendment: Change in Protocol
11/11/08	Safety Report	IND Safety Report
11/11/08	Safety Report	IND Safety Report
11/11/08	General Correspondence	Request for FDA Comment on Study
11/11/08	Maintenance	Request for FDA Comment on Study
11/12/08	TCR	Study Proposal
11/18/08	Safety Report	IND Safety Report
11/19/08	Safety Report	IND Safety Report
11/20/08	General Correspondence	Response to FDA Comments on Protocol
11/20/08	Maintenance	Response to FDA Request for Information
11/24/08	E-Mail	Request for End-of-Phase 2 Meeting Minutes
11/24/08	TCR	Request for FDA Comments on Study
11/24/08	Safety Report	IND Safety Report
12/02/08	E-Mail	Reminder for Request for Feedback
12/02/08	Safety Report	IND Safety Report
12/03/08	Safety Report	IND Safety Report
12/04/08	E-Mail	Status of Request for FDA Comment on Study
12/04/08	E-Mail	Clarification of Request for Trade Name Review
12/10/08	E-Mail	FDA Response

HALAVEN™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
12/10/08	E-Mail	Acknowledgement of FDA Comments
12/12/08	Safety Report	IND Safety Report
12/15/08	Safety Report	IND Safety Report
12/28/08	Safety Report	IND Safety Report
12/29/08	Safety Report	IND Safety Report
01/07/09	Safety Report	IND Safety Report
01/08/09	Safety Report	IND Safety Report
01/12/09	Information Amendment	Updated Investigator's Brochure
01/12/09	Maintenance	Information Amendment: Clinical - Updated Investigator's Brochure
01/20/09	Safety Report	IND Safety Report
01/26/09	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
01/26/09	Maintenance	Protocol Amendment: New Investigator/Updated Investigator
01/27/09	Safety Report	IND Safety Report
01/29/09	Safety Report	IND Safety Report
01/29/09	Safety Report	IND Safety Report
01/30/09	General Correspondence	General Correspondence: Administrative Analysis
01/30/09	Maintenance	Administrative Analysis
02/10/09	Safety Report	IND Safety Report
02/12/09	General Correspondence	General Correspondence: Trade Name
02/23/09	Safety Report	IND Safety Report
02/26/09	Protocol Amendment	Protocol Amendment: New Protocol/New Investigator
02/26/09	Maintenance	Protocol Amendment: New Protocol/New Investigator
03/04/09	Safety Report	IND Safety Report
03/10/09	Safety Report	IND Safety Report
03/18/09	Safety Report	IND Safety Report
03/23/09	Safety Report	IND Safety Report
03/30/09	Safety Report	IND Safety Report
03/31/09	Protocol Amendment	Protocol Amendment: New Investigator/Updated Investigator
03/31/09	Maintenance	Protocol Amendment: New Investigator/Updated Investigator
04/02/09	Incoming FDA Correspondence	Proprietary Name Request
04/06/09	Safety Report	IND Safety Report
04/08/09	Safety Report	IND Safety Report
04/09/09	Protocol Amendment	Protocol Amendment: Change in Protocol
04/09/09	Maintenance	Protocol Amendment: Change in Protocol
04/13/09	Safety Report	IND Safety Report
04/21/09	Safety Report	IND Safety Report
04/30/09	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator/Updated Investigator
04/30/09	Maintenance	Protocol Amendment: Change in Protocol/New Investigator/Updated Investigator
05/14/09	Safety Report	IND Safety Report
05/14/09	Protocol Amendment	Protocol Amendment: Change in Protocol/New Investigator
05/14/09	Maintenance	Protocol Amendment: Change in Protocol/New Investigator
05/20/09	Safety Report	IND Safety Report
05/26/09	Safety Report	IND Safety Report
05/27/09	TCR	Requirements for Treatment Protocols
05/27/09	E-Mail	Requirements for Treatment Protocols
05/27/09	Protocol Amendment	Protocol Amendment: New/Updated Investigators
05/27/09	Maintenance	Protocol Amendment: New/Updated Investigators
05/28/09	General Correspondence	Original Application to establish eCTD Hierarchical Structure
05/28/09	Annual Report	Annual Report 3/31/2808 - 3/30/09
05/28/09	Maintenance	Annual Report 3/31/2808 - 3/30/09

HALAVENT™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
06/18/09	Safety Report	IND Safety Report
06/25/09	E-Mail	Questions on Requirements for Treatment Protocol
06/25/09	Safety Report	IND Safety Report
06/30/09	Safety Report	IND Safety Report
07/06/09	Safety Report	IND Safety Report
07/08/09	Safety Report	IND Safety Report
07/13/09	Safety Report	IND Safety Report
07/15/09	Protocol Amendment	Protocol Amendment: Change in Protocol
07/15/09	Maintenance	Protocol Amendment: Change in Protocol
07/23/09	Protocol Amendment	Protocol Amendment: Change in Protocol/Updated Investigators
07/23/09	Safety Report	IND Safety Report
07/23/09	Maintenance	Protocol Amendment: Change in Protocol/Updated Investigators
07/28/09	Safety Report	IND Safety Report
08/06/09	Safety Report	IND Safety Report
08/18/09	Safety Report	IND Safety Report
08/27/09	Safety Report	IND Safety Report
08/27/09	Request for Meeting	Request for Pre-NDA Meeting
08/31/09	Protocol Amendment	Protocol Amendment: New/Updated Investigators
08/31/09	Maintenance	Protocol Amendment: New/Updated Investigators
09/02/09	Safety Report	IND Safety Report
09/15/09	Safety Report	IND Safety Report
09/21/09	General Correspondence	Sponsor Address Change
09/24/09	Information Amendment	CMC Information Amendment
09/24/09	Safety Report	IND Safety Report
09/28/09	Safety Report	IND Safety Report
09/30/09	Protocol Amendment	Protocol Amendment: Change in Protocol
09/30/09	Maintenance	Protocol Amendment: Change in Protocol
10/01/09	E-Mail	SAP Censoring Rules
10/01/09	Safety Report	IND Safety Report
10/06/09	Safety Report	IND Safety Report
10/07/09	General Correspondence	General Correspondence: Change in Sponsorship/Ownership
10/13/09	Safety Report	IND Safety Report
10/15/09	Briefing Package	Pre-NDA Briefing Package
10/26/09	Safety Report	IND Safety Report
10/28/09	E-Mail	Treatment Protocol Submission
10/29/09	Protocol Amendment	Protocol Amendment: New Protocol/Treatment Protocol
10/29/09	Maintenance	Protocol Amendment: New Protocol/Treatment Protocol
11/04/09	Safety Report	IND Safety Report
11/06/09	Protocol Amendment	Protocol Amendment: New Protocol
11/06/09	General Correspondence	Information Amendment: Clinical
11/06/09	Maintenance	Protocol Amendment: New Protocol
11/11/09	Safety Report	IND Safety Report
11/17/09	Protocol Amendment	Protocol Amendment: New Protocol
11/17/09	General Correspondence	Change in Sponsorship/Ownership
11/17/09	Maintenance	Protocol Amendment: New Protocol
11/19/09	Protocol Amendment	Protocol Amendment: New/Updated Investigators
11/19/09	Maintenance	Protocol Amendment: New/Updated Investigators
11/24/09	Safety Report	IND Safety Report
11/30/09	Safety Report	IND Safety Report
11/30/09	Safety Report	IND Safety Report
12/07/09	Safety Report	IND Safety Report

HALAVEN™ IND 67193 Diligence Log

DATE	TYPE	DESCRIPTION
12/17/09	Safety Report	IND Safety Report
12/21/09	Protocol Amendment	Protocol Amendment: New Protocol
12/21/09	Maintenance	Protocol Amendment: New Protocol
12/22/09	Safety Report	IND Safety Report
01/08/10	Safety Report	IND Safety Report
01/18/10	Safety Report	IND Safety Report
01/22/10	Information Amendment	Protocol Amendment
01/27/10	E-Mail	Official FDA Minutes from 11/23/09 Pre-NDA Meeting
01/27/10	Safety Report	IND Safety Report
01/29/10	Protocol Amendment	Protocol Amendment: New Protocol
01/29/10	Protocol Amendment	Protocol Amendment: New Protocol
01/29/10	Maintenance	Protocol Amendment: New Protocol
02/04/10	Safety Report	IND Safety Report
02/11/10	Safety Report	IND Safety Report
02/16/10	Safety Report	IND Safety Report
02/25/10	Safety Report	IND Safety Report
03/02/10	Safety Report	IND Safety Report
03/16/10	Safety Report	IND Safety Report
03/30/10	Application Submission	New Drug Application [see NDA log]

EXHIBIT 9

HALAVENT™ NDA 201532 Diligence Log

DATE	TYPE	SUBJECT
03/30/10	Application Submission	Original Application
04/02/10	Amendment to a Pending Application	Request for Proprietary Name Review
04/27/10	Incoming FDA Correspondence	ONDQA Project Manager Contact Information
04/28/10	TCR from FDA	Request for information to support sponsor/site inspection
04/29/10	E-Mail from FDA	Request for information related to BIMO inspection planning
05/04/10	E-Mail	NDA 201532
05/04/10	Incoming FDA Correspondence	Request for information
05/05/10	E-Mail from FDA	Request for ECT Waveforms
05/05/10	E-Mail from FDA	Request for information regarding potential BIMO inspections
05/05/10	E-Mail to FDA	Response to request for information regarding potential BIMO inspections
05/06/10	E-Mail from FDA	FDA list of attendees from 5/5/10 FDA teleconference
05/06/10	E-Mail from FDA	Request for Eisai attendee list from 5/5/10 FDA Teleconference
05/06/10	E-Mail to FDA	Change of contact at ICON PLC
05/10/10	T-Con with FDA	Oncology Drugs Advisory Committee Meeting
05/10/10	E-mail	Slope for QTCNi
05/10/10	E-mail	ECG Waveforms
05/10/10	E-mail	Subject slopes for QTCi
05/11/10	Amendment to a Pending Application	Information regarding potential BIMO inspections
05/12/10	TCR from FDA	Site Inspections and Request for Information
05/13/10	TCR	FDA Inspection of Study Sites
05/13/10	E-Mail	Manufacturers of Starting Materials
05/17/10	Amendment to a Pending Application	Response to FDA Request for Starting Materials Manufacturers
05/19/10	Incoming FDA Correspondence	FDA Letter dated 5/13/10 re: starting materials and drug product specs
05/21/10	Amendment to a Pending Application	Submission of ECGs
05/21/10	TCR	Phone Contact with ONDQA Project Manager
05/25/10	TCR	Cancellation of eribulin mesylate Oncologic Drugs Advisory Committee Meeting.
05/25/10	E-Mail	Advisory Committee Meeting
05/25/10	E-Mail	TMF Clarifications
05/25/10	E-Mail	FDA Inspections: Europe
05/25/10	E-Mail	FDA Inspections of ICON PLC and Eisai Limited
05/25/10	E-Mail	NDA Priority Review
05/26/10	E-Mail	Acknowledgment of receipt of email of 5/26/10
05/26/10	E-Mail	Questions & Request for Meeting Regarding Starting Materials
05/26/10	E-Mail from FDA	Written Notification on Cancellation of ODAC
05/27/10	E-Mail from FDA	TMF Clarifications
05/27/10	E-Mail to FDA	TMF Clarifications
05/28/10	E-Mail from FDA	Priority Review Notification
06/02/10	E-Mail to FDA	Requested site documents for FDA inspection
06/03/10	E-Mail from FDA	FDA Announcement of EU Inspections
06/03/10	TCR from FDA	Inspection of U.S. study sites
06/03/10	E-Mail from FDA	Requested site documents for FDA inspection
06/03/10	E-Mail to FDA	European Inspection Site Travel Information
06/04/10	E-Mail from FDA	Information Request: Datasets
06/04/10	E-Mail to FDA	Confirming receipt of email (Information Request - Datasets)
06/04/10	E-Mail to FDA	Status of package (background information for FDA

HALAVEN™ NDA 201532 Diligence Log

DATE	TYPE	SUBJECT
		inspections)
06/07/10	Amendment to a Pending Application	Quality Information Amendment - Response to FDA Request
06/07/10	TCR	Starting Materials
06/10/10	E-Mail from FDA	Confirmation of Background Information for Site Inspections
06/10/10	TCR to FDA	Request to FDA re: 6/4/10 FDA Information Request
06/10/10	E-Mail to FDA	Confirmation of Background Information for Site Inspections
06/11/10	E-Mail from FDA	NDA Filing Communication
06/11/10	E-Mail to FDA	Eisai Response to 6/4/10 FDA Request
06/11/10	E-Mail to	Eisai responses to 6/4/10 FDA Information Request (Clinical)
06/12/10	Incoming FDA Correspondence	Starting Materials Letter dated 6/9/10
06/16/10	E-Mail	FDA General Advice Letter of 6/9/10
06/17/10	Amendment to a Pending Application	Information Request -Datasets
06/22/10	Request for Meeting	Meeting Request Correspondence – Type C
06/22/10	E-Mail	Request from Inspectors
06/22/10	TCR	Starting Materials
06/22/10	TCR	Starting Materials
06/22/10	TCR	Starting Materials
06/22/10	E-Mail	Meeting List of FDA Attendees
06/23/10	E-Mail	Letter Granting Meeting Request
06/23/10	TCR	Meeting Agenda and Preparation
06/23/10	E-Mail from FDA	FDA Information Request: Clin. Pharm
06/29/10	Request for Information	Response to FDA 74-Day Review Request
06/29/10	E-Mail	Response to FDA 79-Day Review Letter
07/01/10	E-Mail from FDA	Response to Arrangements for Site Inspections in France and Spain
07/01/10	E-Mail to FDA	Arrangements for Site Inspections in France and Spain
07/06/10	E-Mail	FDA Acceptance of HALAVEN Trade Name
07/08/10	E-Mail	Starting Materials
07/09/10	Incoming FDA Correspondence	FDA Information Request Letter (CMC) dated 7/2/10
07/15/10	TCR	Call to FDA
07/15/10	E-Mail	FDA Comments: HALAVEN Carton and Vial Labels
07/19/10	E-Mail	Submission Plan and Teleconference Request (CMC)
07/22/10	TCR	FDA Contact CMC
07/22/10	E-Mail	Submission of revised draft vial and carton labels per FDA e-mail request dated 7/14/10
07/23/10	Response to FDA Request	Submission of revised vial and carton labels per FDA 7/14/10 request
07/28/10	Response to FDA Request	120-Day Safety Update and Updated Survival Analyses
07/28/10	Amendment to a Pending Application	Quality Information Amendment to a Pending Application: Responses to FDA Information Request
07/28/10	E-Mail	HALAVEN Carton Label
07/29/10	E-Mail	FDA Information Request (CMC)
08/03/10	E-Mail from FDA	Confirmation of Addresses for EU sites
08/03/10	E-Mail	Clinical Review of Study
08/06/10	E-Mail	Response to FDA Information Request - Microbiology
08/09/10	Amendment to a Pending Application	Responses to FDA Information Request
08/09/10	Amendment to a Pending Application	Response to FDA Information Request dated 7/29/10
08/12/10	Amendment to a Pending Application	Amendment to a Pending Application Information Request

HALAVEN™ NDA 201532 Diligence Log

DATE	TYPE	SUBJECT
08/18/10	TCR	Pediatric Advisory Committee Meeting
08/19/10	E-Mail	Clinical Information Request
08/19/10	E-Mail	HALAVEN Pediatric ODAC
08/31/10	Incoming FDA Correspondence	FDA Information Request Letter CMC dated 8/30/10
09/01/10	E-Mail	Confirmation of Teleconference for 9/3/10
09/02/10	E-Mail	Background Materials for Teleconference on 9/3/10
09/07/10	Incoming FDA Correspondence	FDA Minutes of 7/2/10 Meeting Regarding Starting Materials
09/08/10	E-Mail	NDA 201532
09/08/10	Incoming FDA Correspondence	Eribulin NDA Review Extension
09/08/10	E-Mail	Post Marketing Requirements and Commitments
09/09/10	E-Mail	Post Marketing requirement
09/10/10	Response to FDA Request	Response to 6/23/10 FDA request
09/10/10	General Correspondence	Minutes of Eisai-FDA meetings on 7/2/10 and 9/3/10
09/14/10	E-Mail	FDA-Carton and Vial Label Revisions
09/14/10	TCR	End-of-Phase-2 Meeting
09/14/10	E-Mail	Post marketing requirement and commitments
09/15/10	E-Mail	Study Duration
09/16/10	E-Mail	Pediatric ODAC Meeting Information
09/16/10	E-Mail	Eribulin Pediatric ODAC Update
09/17/10	E-Mail	NCI Study
09/20/10	E-Mail	HALAVEN Revised Carton and Label
09/20/10	E-Mail	Postmarketing Commitment #2
09/22/10	E-Mail	FDA Comments on PI and patient PI
09/23/10	E-Mail	Post marketing requirement
09/27/10	E-Mail	HALAVEN Carton and Vial Label
09/28/10	E-Mail	Revised Carton and Vial Label
09/28/10	E-Mail	HALAVEN PI and Patient PI
09/29/10	E-Mail	Postmarketing Requirement #1
10/04/10	E-Mail	CMC Postmarketing Commitment
10/04/10	E-Mail	CMC Postmarketing commitment
10/05/10	E-Mail	PI Content
10/07/10	TCR	PI Content
10/08/10	E-Mail	HALAVEN Carton and Vial Labeling
10/08/10	E-Mail	Response to FDA request of 10/5/10
10/08/10	E-Mail	Briefing Package For HALAVEN Pediatric ODAC Meeting on 11-30-2010
10/08/10	TCR	HALAVEN Carton and Vial Labeling Changes
10/12/10	E-Mail	HALAVEN Carton and Vial Label Revisions
10/13/10	E-Mail	Post Action Feedback Meeting with FDA on HALAVEN NDA Process
10/18/10	E-Mail	HALAVEN Carton and Vial Label
10/18/10	E-Mail	FDA Comments on PI and PPI
10/19/10	E-Mail	HALAVEN Carton and Vial Label Revisions
10/20/10	E-Mail	Finalization of HALAVEN Vial and Carton labeling
10/20/10	E-Mail	HALAVEN Package Insert and Patient Package Insert Revisions
10/26/10	TCR	Pending FDA Comments on 10/20/10 Submission
10/26/10	E-Mail	Post Action Feedback Meeting On HALAVEN NDA Process
10/28/10	E-Mail	HALAVEN Final Labeling: PI and PPI
10/28/10	E-Mail	FDA Comments on 10/22/10 Labeling Submission
10/28/10	E-Mail	Final Draft Labeling
11/05/10	E-Mail	HALAVEN PI Revisions
11/05/10	E-Mail	HALAVEN PI Revisions

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DATE	TYPE	SUBJECT
11/10/10	E-Mail	Final PI Revisions
11/10/10	E-Mail	HALAVEN PI Revisions
11/16/10	E-Mail	HALAVEN FDA Approval Letter
11/16/10	E-Mail	Postmarketing Commitment 1689-#1
11/18/10	E-Mail	Revised HALAVEN Approval Letter
11/28/10	E-Mail	Request for Patent Information (FDA Form 3542)
12/06/10	Incoming FDA Correspondence	Requests for Additional information: Clinical Pharmacology
12/09/10	E-Mail	Submission of Final Printed Carton and Container Labels